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HERON AND EGRET LOSSES DUE TO VERMINOUS PERITONITIS

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OWEN[5] has shown that herons have a high death rate during the first year, but the causes of death, other than shooting or starvation, are largely unknown. A contributing factor may be *Eustrongylides ignotus*, a common parasite in the peritoneal cavity. Jagerskiold[4] first described this nematode from bitterns (*Botaurus* sp.) in Europe. Chapin[2] later described its occurrence in the great blue heron (*Ardea herodias*), and Cram[3] reported it from the black-crowned night heron (*Nycticorax nycticorax*); both in the United States. Bowdish[1] reported fatal cases of verminous peritonitis in a great blue heron and in a black-crowned night heron. This paper describes a second fatal case in a great blue heron and reports the first fatal case and the first known occurrence of the parasite in the American egret.

CASE I

On July 29, 1958, two wardens of the Maryland Department of Game and Inland Fish brought a great blue heron to the Disease Laboratory of the Patuxent Wildlife Research Center for examination. The bird had been found lying moribund in the mud along a small tidal creek.

The bird was an emaciated immature male. The pectoral muscles were shrunken and the sternal keel was very prominent. The heart was enlarged and flabby. The proventriculus contained only a few dragonfly fragments.

The ventral portion of the peritoneal cavity was filled with a shieldlike mass of coiled fibrous tubes containing large red nematodes, adhered intestines, and ingesta. The intestines were so tightly entwined with the fibrous tubes that they could not be separated without cutting both worms and

intestines. Several attempts were made to remove the worms from the fibrous tubes, but the nematodes were so entwined that most specimens broke. Three that were removed for identification proved to be *Eustrongylides ignotus*. Two nematodes had burrowed through the liver parenchyma, the coils of the worms looping out of the liver and re-entering several times. There was no fibrous attachment of the worms to the liver, and the worms were easily withdrawn. Material from lesion and liver sections was fixed, mounted, and prepared in the usual manner, staining with Harris' haematoxylin and eosin.

Microscopically, the liver sections from which the worms were removed showed only an area of hemorrhage, fibrin strands, and acellular eosinophilic material. A few fibroblasts were at the edge of the lesion. There were no fibrous tubes in the liver, and the histologic picture suggests that the worms had penetrated the liver parenchyma shortly before the death of the heron.

Sections of the peritoneal lesion showed that the nematode was in a highly vascular fibrous tube (the result of the inflammatory response of the heron) lying on the serosa of the intestine. The outermost cellular layer of the tube consisted of connective tissue with some large veins and a few arteries. Next came a layer of fibrous tissue containing many small venous sinuses. Closer to the nematode there was cellular infiltration of this vascular area. The infiltrating cells were primarily granulocytes, with a few mononuclear cells. The most conspicuous cells in this zone, however, were the nucleated erythrocytes that packed the sinuses. Next, there was a small zone of increased fibrous tissue that was frequently fragmented. In some sections, venous sinuses appeared to open directly into the zone nearest the worm. Immediately adjacent to the worm was a zone of necrotic cellular debris, composed mostly of karyolytic erythrocytes, nuclear fragments, and necrotic cells.

One section showed the remnants of a tube that had penetrated the muscular layers. The lumen of this tube contained clumps of an acellular acidophilic mass with nuclear fragments. The lining was a rigid-appearing, eosinophilic,

layered wall containing several rows of basophilic nuclear fragments. Bordering this was an area containing necrotic cells, surrounded by a zone of fibroblasts. Three nearby arteries appeared to be undergoing destruction. The media contained an amorphous grayish-pink substance that increased in thickness, finally closing the lumen. This material may be amyloid in nature; however, no special stains were used.

CASE II

On October 30, 1959, two American egrets (*Casmerodius albus*) from the Bombay Hook National Wildlife Refuge in Delaware were submitted to the Patuxent Wildlife Research Center for examination. The first had been kept in a freezer

Fig. 1. Verminous peritonitis in an American egret, due to *Eustrongylides ignotus*.

for a week after its death. Two specimens of *Eustrongylides ignotus* were found, but the cause of death was not determined. The second bird, an adult female, was captured alive at the edge of a pond, trying to hold its head above the water. This egret exhibited the same severe verminous peritonitis described in the previous case.

The heart contained a large chicken-fat clot. The trachea and bronchi contained frothy white fluid. There were small hemorrhages in the muscles near several of the neck vertebrae. The proventriculus contained only grass fragments. The peritoneal cavity contained a large shield-shaped mass 5 cm long and 4 cm wide at its greatest width (Fig. 1). The structure of the mass was identical with that described from the great blue heron. One of the *Eustrongylides ignotus* removed from this mass was 13 cm long. None of the nematodes had penetrated the liver, although the coiled tubes had made impressions on the liver surface.

SUMMARY

This paper records the second case of fatal verminous peritonitis reported in a great blue heron, and the first in an American egret. *Eustrongylides ignotus* had not previously been reported from the American egret.

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"CELL AGGLUTINATION" TECHNIQUE FOR QUANTITATIVE TITRATION OF FOWLPOX VIRUS*

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"POCK COUNTING" has been the primary method used for titrations of fowlpox virus in infected material. However, the accuracy of counting the pocks on the chorioallantoic membrane (CAM) of embryonating eggs has been seriously questioned by some research workers[1,2] and is cumbersome. The critics have stated that there is a "wide scatter" in the count of individual dilutions and that the results do not follow Poissonian or normal distribution. Dulbecco's "plaque" technique[3] has not been found suitable for quantitation of fowlpox virus, for the "pinpoint" plaques produced by this virus are not easily detected by the naked eye. There is urgent need for an accurate and simple technique for quantitative analysis of pox viruses.

Recently, Mayyasi *et al.*[4] reported that L cells (Earle) of mouse fibroblasts were agglutinated by vaccinia virus. L cells were used to develop agglutination and agglutination-inhibition titration methods for vaccinia virus.

In the following technique, chicken embryo cells (CE cells) were found suitable for developing an accurate method for estimation of viral concentration in fowlpox-infected tissue-culture fluids.

Materials. Chicken embryo (CE) cell suspensions were prepared by the standard technique.[3] Seven-day-old chicken embryos were found preferable to older ones (9–10 days) as

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the source of cell suspensions since trypsinization required less time and single-cell suspensions were obtained with fewer cell clumps and a greater proportion of epithelium-producing cells than fibroblasts.

Chicken embryos were minced with scissors and trypsinized with 0.25% trypsin (Difco, 1:250) in Hank's solution at 37°C. The minced chicken embryo tissue was rapidly drawn in and out of 10-ml pipettes to separate the cells. The suspension was then centrifuged for 30 minutes in a cold room (4°C) at 600 rpm, and the cells of the sediment were suspended in the growth medium that was used for the "cell agglutination" titrations. The growth medium consisted of Hank's balanced salt solution, 96%; inactivated bovine serum, 3%; lactalbumin hydrolysate, 0.5%; and standard antibiotic solution, 0.5%.[3] The strain of fowlpox virus used in this work was isolated by Doll in 1950.[5] Fowlpox virus used in both tissue cultures and on the CAM was employed. Two tissue-culture passages of fowlpox virus were used. They are identified as FPV/TCII, indicating the second tissue-culture passage, and as FPV/TCIII, indicating the third tissue-culture passage.

Procedure: The CE cells, suspended in the growth medium, were standardized by direct count of the cells in a hemocytometer, and the final suspension contained 1,000,000–2,000,000 cells/ml. This concentration produced the best "cell agglutination."

The material to be tested for fowlpox virus was serially diluted (2-fold) in the growth medium. In each test, duplicate dilutions were used and the viral samples were tested at different times. Agglutination tubes of Wasserman or Kahn type were used for making serial dilutions of the viral suspensions. To each tube, containing 0.9 ml of the virus dilution, 0.1 ml of the CE cell suspension was added and the suspensions were mixed thoroughly. The tubes were then tightly stoppered and incubated in slanted position for 36–48 hours at 37°C. After incubation, the agglutination pattern of the CE cells was viewed under a microscope at a magnification of 100×.

The pattern of the CE cells completely agglutinated by

fowlpox virus is shown in Fig. 1. Incomplete clumping of CE cells was seen when the concentration of the virus in the suspension was decreased. Although some clumping of cells was observed within two hours of inoculation, 24–48 hours was found most suitable and accurate.

Table 1 presents the titrations of fowlpox virus in the infected CAM and in the supernatant fluid of the infected tissue cultures. The "cell agglutination" titer of the fowlpox virus increased after 2 passages in tissue culture. The fowlpox viruses grown in tissue culture FPV/TCII and FPV/TCIII caused agglutination in the 1:2560 dilution, which represented

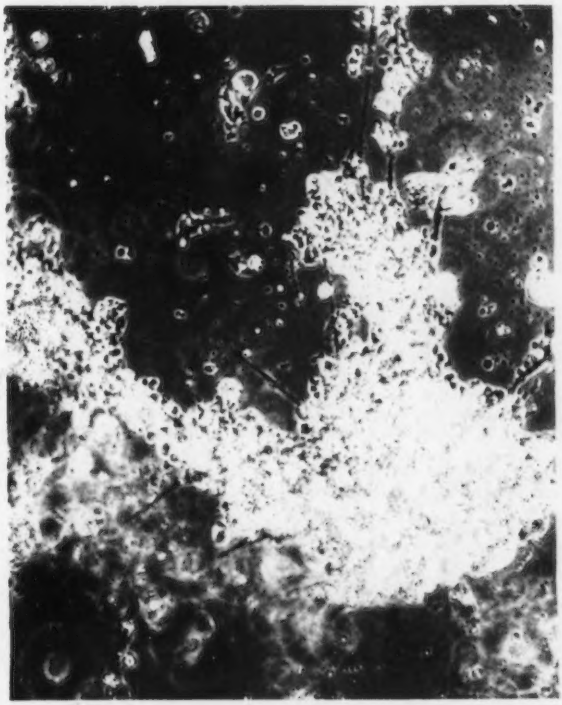

Fig. 1. Agglutination of chicken embryo fibroblasts 24 hours after inoculation of cell suspension with fowlpox virus.

Table 1. "Cell agglutination" titers of tissue culture fluids from the two and three passages of virus in CE cells and a chorioallantoic membrane suspension.

Virus dilution	Cell agglutination titer[1] at 48 hours				
	FPV/TCII[2]	FPV/TCIII[3]	CAM susp. seed control virus	Normal tissue culture fluid	FPV heated 56°C for 15 min.
1:1	+	+	+	—	—
1:10	+	+	+	—	—
1:20	+	+	+	—	—
1:40	+	+	+	—	—
1:80	+	+	+	—	—
1:160	+	+	+	—	—
1:320	+	+	+	—	—
1:640	+	+	±	—	—
1:1280	+	+	±	—	—
1:2560	+	+	—	—	—

[1] +, marked cell agglutination; ±, cell agglutination in abscessation; —, normal growth of cells.
[2] FPV/TCII, fowlpox virus tissue culture passage 2.
[3] FPV/TCHIII, fowlpox virus tissue culture passage 3.

2560 "cell-agglutinating units" per 0.1 ml (reciprocal of the highest dilution showing definite clumping). The fowlpox virus in the CAM suspension gave a titer of 1280 "cell-agglutinating units."

Reproducible results were achieved by the "cell agglutination" technique when the samples were titrated at different times within several weeks. The test gave a more consistent estimation of the viral content of a fluid than did pock counting on the CAM. The technique was simple and economical. A chicken embryo usually yielded sufficient cells for 50 agglutination tubes. Furthermore, less time was required than for the pock counting technique since the results of the cell agglutination test could be determined within 48 hours.

SUMMARY

It appears that the "cell agglutination" technique can be used advantageously for quantitative titration of pox viruses. It is simple, economical, accurate, and less time-consuming than the "pock counting" technique.

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CASE REPORT: HOCK SWELLINGS AS A PRIMARY LESION OF SALMONELLA PULLORUM INFECTION IN BROILER CHICKENS

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Recieved 23 September 1960

WITHIN two months 9 cases were submitted to the diagnostic laboratory in which the principal complaint was a high incidence of lameness due to swollen hocks. All cases involved broiler chickens 3–4 weeks old, coming from several different chick sources. Losses to disease could not be established, for early chick mortality was only slightly above normal in these flocks, though several of them contained a considerable proportion of stunted birds. No medication was given before the outbreaks or diagnosis.

On post-mortem examination the majority of the birds had no lesions other than articular and periarticular swellings at the hock joints. These swellings were either unilateral or else far more pronounced on one side than on the other. An occasional bird had a swollen foot-pad in addition to the hock lesion. The joint capsule was swollen, and the articular cavity contained creamy-white to yellow fluid. The periarticular swellings consisted of edematous tissue rather than free fluid. In one bird the distal portion of the tibia was enlarged on the affected side. A few birds from each flock exhibited some of the more orthodox internal lesions of pullorum, including nodules in the liver, gizzard, and heart, and an excess of pericardial fluid that was either turbid or contained floccular material.

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Fig. 1. Articular and periarticular swelling of the hock joint of a four-week-old broiler chicken. *S. pullorum* was isolated from the joint fluid.

Fig. 2. Swollen foot-pad of a four-week-old broiler. The hock is also swollen on the affected side. *S. pullorum* was isolated from the foot-pad and liver of this bird.

Salmonella pullorum was isolated from all of the lesions in these cases. Fluid was obtained aseptically from the joint swellings, and the liver, heart, and gizzard lesions were cultured with a hot platinum loop after the surface of the organ had been seared with a red-hot spatula. The inoculum was then streaked on differential Salmonella-Shigella agar. Small colonies grew within 24–48 hours, and the color of the medium was changed from red to yellow. A colony picked from this agar fermented glucose, producing acid and gas, but did not ferment lactose, sucrose, dulcitol, or urea. In triple-sugar-iron agar slants a red slant, black zone, and a yellow butt were produced. These reactions are typical of *Salmonella pullorum*, and subcultures were identified as *S. pullorum*, 9-12, at the Communicable Disease Center of the U. S. Public Health Service, Chamblee, Georgia.

These cases are reported because, although *Salmonella pullorum* has been previously isolated from sporadic cases of arthitis in chickens,[2,3,4] this type of arthritis and periarthritis has not been previously reported as a flock problem. It was particularly striking that 9 flocks of diverse origin should be afflicted with this manifestation of pullorum disease within such a short period and a limited geographical area.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. S. Cover for help in preparing this report, and to Dr. P. R. Edwards for identifying and typing the bacterial cultures.

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SALMONELLA AND ARIZONA CULTURES FROM AGRICULTURAL SOURCES: 1959

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DURING 1959, 2,350 cultures of *Salmonella* isolated from agricultural sources in the United States were typed serologically at the Communicable Disease Center, Chamblee, Georgia. This was an increase of 619 over the previous year.[1] The cultures are summarized in Table 1. In addition, there were 95 cultures belonging to the Arizona group, making a total of 2,445 cultures in these two closely related groups of organisms.

Of the 2,350 salmonellae identified, 1,729 (73.6%) were from animals. As might be expected, by far the largest proportion of the strains from animals were of avian origin (70.5%); turkeys accounted for 33.5% and chickens for 33.8%. In previous years, cultures from turkeys have far outnumbered those from chickens;[1,2] in 1958, 57% of the Salmonella cultures serotyped were from turkeys and 29% were from chickens.[1] Over one-quarter of the cultures from animals came from cattle (8.9%), swine (8.6%), and horses (8.7%) in contrast with previous years, when only 7.5% of the animal strains were from all domestic farm animals combined.

A rather large number (140) of strains from pork and pork sausage were received from one investigator; this was 5.9% of the total salmonellae received. There was an increase in the proportion of cultures from animal feeds and feed ingredients—from 11% of the total in the previous year[1] to 20%. This reflects the current concern about contaminated

* With Laboratory Services, Animal Disease Eradication Division, ARS, USDA; assigned to the Enteric Bacteriology Unit, CDC, since April, 1957.

Table 1 Distribution of Salmonella serotypes (numbers of isolations)

	Turkeys	Chickens	Ducks	Geese	Pigeons	Pheasants	Quail	Other Birds	Swine	Cattle	Sheep	Horses	Cats	Dogs	Mice and Rats	Guinea Pigs	Other Mammals	Reptiles	Milk	Powdered Eggs	Pork and Pork Scraps	Dog Food	Livestock and Poultry Feed	Meat and Meat Scrap Materials	Fish Meal	Bone Meal	Feather Meal	Poultry By-products	Garbage	Pet	Drainage	Cotton and Soy Bean Meal	Total
alachua	2																						1	4								7
anager		1																															1
anatum	54	18	1				4		4	6		3		1				1			17		4	1			1			5		120
bareilly		3																									2						8
berta		1																															1
blana	8	4							1						1							5	1	8						4		34
blockley	1	36							3															1									41
b-rip-morbificans	2										1																						3
braenderup	1	2																															4
bredeney	17	4							1	3					2								1	1			1	6		2			57
california	8	4												3										6									21
carno		3																						5	2		1						12
champaign																						1		5									5
chester	37																							1									38
chelarae-mis																																	
dar. Rosenmuller		2							93	3																							98
cubana	8								1																								1
denver	1																						1	12						1			14
derby	22	9							25	1	1			1			1				99	2	1	6		1				2	2	173
dublin		2								8		2												1									13
enteritidis	10	14				1				47		38			3	1								3									172
gallinarum	3	75																															78
gaminara																								3									3
give	6	1																															7
hartford										1																							1
heidelberg	33	42		1			1			1														17						4			97
illinois									1			1		2			2					2		16						4			28
indiana	1	4											1																	1			5
infantis	28	21																															79
java	2				1																												4
javiana																																	1
johannesburg																								2									2
kentucky	1	4										1										3		9			1			3			18
lexington																								10			6	2		3			18
litchfield	1																										2						20
litchfield																																	1
london									1																								2
madella	1																																1
manila																								2									2
meleagridis	4		1							1												2		1									3
miami										1							1						1	1						1			3
minnesota										1				1																			2
mississippi									1					1										2			1			2			4
montevideo	1	6			1				4	1		2		1			4					2	2	30	2		1	4		4	1		47
muenchen	44	3								1					1									1									51
new brunswick	1																					2											3
newington	3	2								1														1									4
newport	44	4								24	1	3		1			1				24	1		1					2	1			106
ohio										1														2									5
oranienburg																								28						2			31
panama	4																							2									6
pullorum	2	194						3																									2
reading	4	1																		3													197
st-paul	28	4										1												3									8
san-diego	12	7																															36
schwarzengrund	13	1																						12			1			4			26
senftenberg	8								1															19	1		2			10			31
siegburg													1									3	5				1	3		1			49
simsbury																							2	2									5
takoma																																	3
tennessee	5	9																				5		2			2						9
thomasville																							1	19						1			21
thompson	5	3								1	1																	2						35
typhi-murium	137	89	1	12	21			7	9	52		38	1	4	6	1	1	6	1		3			4			1						2
typhi-suis									1															3						2			18
urbana																																	394
westhampton																								1						2			1
worthington	7	5																						4			2			2			3
4,12:e,h	1																					2		14			9	2		4	1		8
4,3,12:non-motile	2														1	2																	44
1,13,23:non-motile	1																																1
TOTAL	530	584	3	14	22	1	5	10	148	154	3	151	3	15	14	4	16	2	1	6	140	29	23	284	6	1	31	26	2	1	67	4	1
																																	2,350

Table 2. Distribution of Arizona Serotypes

		Turkeys	Chickens	Sheep	Reptiles	Total
ARIZONA	1,2: 13,14		2			2
"	1,33 23-21				1	1
"	5: 1,7,8	1				1
"	7: 1,2,6	55	1			56
"	7: 1,7,8	28	2			30
"	10: 1,2,5,6				1	1
"	10: 17,20	1				1
"	16: 22-21		1			1
"	26: 29-30			1		1
"	26: 32-31				1	1
TOTAL		85	6	1	3	95

animal feeds and their effect on the health of poultry and livestock in this country.

Sixty-eight different *Salmonella* serotypes were recognized. The greatest variety was found in animal feeds and feed ingredients—46 different types. Thirty-nine different types were seen from turkeys, 33 from chickens, 16 from swine, 18 from cattle, 10 from horses, and 9 from dogs. *S. typhi-murium* was the serotype with the widest distribution. It made up 22% of all the strains from animals. *S. pullorum*, which was largely confined to chickens, made up 11.4% of the total animal strains. *S. enteritidis*, formerly quite uncommon in the United States, made up 10.2% of the strains from animals.

The 95 cultures of the Arizona group are summarized in Table 2. All of these cultures were from animals (89.5% from turkeys).

ACKNOWLEDGMENT

Acknowledgment is made to Dr. P. R. Edwards, in whose laboratory at the U. S. Public Health Service, Communicable Disease Center, Chamblee, Georgia, these cultures were serotyped. Thanks are also due to Mrs. Carolyn Ramsey, who shared in the work of typing the cultures.

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SUSCEPTIBILITY OF PLEUROPNEUMONIA-LIKE ORGANISMS TO BENZALKONIUM CHLORIDE

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CERTAIN pleuropneumonia-like organisms (PPLO) are considered to be the etiologic agent for Chronic Respiratory Disease (CRD) of chickens.[1]

The susceptibility of PPLO strains to the action of numerous antibiotics and chemical compounds has beeen studied by several workers.[4,5,7,12]

Preliminary work in our laboratory indicated that benzalkonium chloride has a wide antiseptic range against bacteria and fungi as well as PPLO without affecting such strains of Newcastle disease virus as B_1, Roakin, LaSota, and Queretaro. This suggests the possible use of BC to ensure Newcastle disease virus vaccines free of contamination with PPLO, bacteria, or fungi.

This paper reports on the susceptibility *in vitro* of some strains of PPLO from avian origin to the action of a quaternary ammonium compound: benzalkonium chloride (BC). This BC salt has the following formula: para-di-isobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride. This cationic detergent has antiseptic qualities and it is considered as a surface-active agent because it lowers the surface tension of solutions.[9]

MATERIAL AND METHODS

Two PPLO strains recently isolated from clinical cases of CRD in fowls were used. Both were isolated from the trachea and lung of infected birds in different outbreaks. They have been designated PP and C-PE strains.

Modified Grumbles' liquid medium (GLM),[6,10,11] 10 ml in each tube, was used as the isolation medium and in the experimental work.

Fermentation reactions of both strains were determined in dextrose, maltose, sucrose, and dulcitol.[2,3,8,13] Phenol red broth (10 ml) with 1% Difco PPLO serum fraction and 0.5% of one of the above-mentioned carbohydrates was used.

The smears of both strains from the sediment of centrifugated cultures were stained by Giemsa's and Gram's methods. Each PPLO strain was seeded in a flask containing 50 ml of GLM and incubated 12 days. The flask cultures were tested for bacterial contamination with tryptose broth, fluid thioglycollate medium, and Sabouraud dextrose agar. The BC was diluted (w/v) with buffered saline solution pH 7.2 from 1:1000 to 1:4,096,000 (twofold dilutions); 1 ml amounts of each dilution were distributed in sterile glass tubes.

One ml of the PPLO flask cultures was added to each tube of BC dilutions using two different series for each strain, and the two series of tubes were incubated in separate racks in 20 and 37°C water baths. Glass tubes containing one ml of a single PPLO culture were incubated as controls at each temperature.

Each tube containing BC plus PPLO mixture and controls from each strain were seeded with a wire loop in different tubes with GLM, immediately and 15 and 30 minutes after mixing. All seeded tubes were incubated at 37°C as long as 21 days.

Both PPLO strains were titrated by using tenfold dilutions from the flask cultures mentioned above, using GLM as diluent. Each dilution was seeded with the same wire loop in a different GLM tube, and all were incubated 21 days at 37°C. The titer of each strain is the greatest tenfold dilution from which reisolation was successful after 21 days of incubation.

Acid production (yellow color) in the GLM tubes was taken as evidence of growth of PPLO strains.

RESULTS

Both strains are slow-growing (yellow is usually apparent

after 10 days' incubation) and have similar fermentation reactions. Both strains made use of dextrose, maltose, and sucrose (delayed fermentation), but not of dulcitol. The sterility tests showed no bacterial or fungal contaminations. The Giemsa-stained smears showed coccoid bodies in single and cluster forms.

Published titers of PP and C-PE strains are 10^{-5} and 10^{-3}, respectively.[4,6]

In vitro, BC showed effective action in inhibiting the growth of PPLO strains. Activity against them varied from 1:16000 to 1:512,000, changing with strain, temperature, and time of incubation. Table 1 shows the results.

Table 1. Maximum dilution of benzalkonium chloride that showed red color inhibition of PPLO after 21 days' incubation.

Incubation		Strain	
Time (minutes)	Temperature (C)	PP	C-PE
0	20	1:16000	1:128000
15	20	1:32000	1:128000
30	20	1:64000	1:512000
15	37	1:32000	1:256000
30	37	1:64000	1:512000

DISCUSSION

The results demonstrate that BC is able to inhibit the growth of PPLO strains. We do not know what type the strains are, but we suppose they belong to the pathogenic group (group I) because of their characteristics: slow growth,[3] biochemical behavior,[3,8,10,13] morphology.[3,6,13]

It is obvious that the action of BC against PPLO strains is directly related to time and temperature of incubation, titer of the PPLO strain, and BC concentration.

SUMMARY

Different dilutions of benzalkonium chloride were tested in vitro for inhibition of two PPLO strains, varying the temperature and time of exposure. The growth of both strains was inhibited.

ACKNOWLEDGMENT

The author appreciates the technical assistance of Miss Rosa Maria Tapia.

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ORIGIN OF S6 STRAIN MYCOPLASMA

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THE origin of the S6 strain Mycoplasma was first reported at the 4th Annual Western Poultry Disease Conference, Davis, California, February, 1955. The proceedings were not published. This strain has become widely used in research work with Mycoplasma.[1,2,3,4,5,6,7] With such widespread reference to the strain, it seems appropriate to clarify its origin.

On June 1, 1954, a turkey grower in the central San Joaquin Valley of California reported a sinusitis outbreak in a group of eight-week-old turkeys of a Broad-Breasted White strain. The owner had two turkey-rearing houses, about 50 feet apart, with outside runs. Each house held about 2000 turkeys. At the time of the disease outbreak one house held Broad-Breasted Bronze poults from one breeder source, and the other house held the white strain, from another breeder source. Both groups were about 8 weeks old when the writer observed the condition. Poults of the Broad-Breasted Bronze strain showed no signs of the disease, whereas the poults of the white strain were severely affected, exhibiting swollen sinuses, lacrimation, and coughing. In addition, about 5% of the birds were showing signs of involvement of the central nervous system. In addition to uncoordinated movements many affected turkeys held their heads with one side turned slightly upward.

Typically affected poults were selected by the writer and taken to the laboratory at Davis, where, in addition to isolations from the tracheas, air sacs, and sinuses of several turkeys, Mycoplasmas were isolated from the brain of one show-

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ing nervous signs. Hemagglutination-inhibition tests for Newcastle disease were negative.

A broth culture of the Mycoplasma was inoculated intracerebrally, intravenously, or into the sacral sinus of the spinal canal of 4 three-week-old poults. All four died. Cultures of Mycoplasma from the brains of the inoculated poults were grown in noninhibitory media, pooled, and inoculated into 6 more young poults: 1 intratracheally, 1 intravenously, 2 intracerebrally, 1 in the infra-orbital sinus, and 1 in the sacral sinus of the spinal canal. The poult that received the intratracheal inoculation developed no symptoms during the eight-week observation period. The one inoculated in the sacral sinus developed pronounced respiratory signs within 6 days. The one inoculated in the infra-orbital sinus developed sinus swelling within 3 days. Another one inoculated intravenously began to show cerebral disturbances (wobbly, unsteady gait) within 3 days, and these signs continued throughout the eight-week observation period, becoming progressively more severe. Distinct lower-respiratory signs were obvious within 22 days. One of the poults inoculated intracerebrally never developed detectable signs of any kind, but the other one showed some depression by 5 days and a wobbly gait was noticed by the twelfth day post-inoculation. These abnormal locomotor symptoms were usually detected only when the poults were disturbed. The signs of a central nervous system infection appeared sooner and seemed more severe in the poults inoculated intravenously than those inoculated intracerebrally.

Isolated from the brain of a naturally infected turkey was a virulent strain of Mycoplasma that caused nervous symptoms and locomotor disturbances as well as respiratory signs when reinoculated into turkeys. It was thereafter subcultured in the laboratory as the writer's sixth stock culture and named "S6."

SUMMARY

The origin of S6 strain of *Mycoplasma gallisepticum* is noted, together with the first observed evidence that the strain had neurotropic tendencies.

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THE EFFECTS OF RAPID EMBRYO PASSAGE UPON THE INFECTIOUS BRONCHITIS VIRUS[1,2,3]

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IT HAS been well established that the virus of infectious bronchitis (IB) of chickens changes its immunogenic properties upon repeated embryo cultivation.[5,8,9] It is equally well known that the Beaudette strain 42 of virus, when propagated in embryonating chicken eggs, has been prone to develop titers of $10^{-5.0}$ to $10^{-7.0}$, which makes end-point titrations of immune sera impossible without resorting to a diluted serum method.

This investigation was conducted to determine whether rapid embryo passage [i.e., passage of the infectious bronchitis virus (IBV) at the peak of the logarithmic phase of growth] would change the characteristics of a virulent strain of virus in regard to virus concentration, embryo mortality rate, and pathogenicity for the host. In addition, it was expected that rapid embryo passage of the Beaudette strain 42 of IBV would result in an increase of the EID_{50}.

IBV was first reported by Beaudette and Hudson[1] to be readily propagated in embryonating chicken eggs. Continued serial passages of the virus in embryos increased the virulence of the virus for embryos and was accompanied by a decrease in virulence for chickens.

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Delaplane and Stuart[5] reported modification of the virus as a result of propagation in chicken embryos. Passage of the virus required 2 years in order to achieve 90 weekly embryo passages.

Hitchner and White[7] studied the growth rates of an egg-adapted IBV strain and non-egg-adapted strains, and found no significant differences in virus concentrations of the harvested material when various dilutions of virus were inoculated. The log phase of growth of the non-egg-adapted strains occurred 6 hr. postinoculation, with maximum virus concentration attained after 24–30 hours. An embryo-adapted virus was in the log phase 4 hr. postinoculation, with maximum concentration occurring after 12 hr incubation.

Chomiak *et al.*[2] demonstrated a cytopathogenic effect (CPE) in tissue culture with the Beaudette strain during the second passage of the virus in chicken embryo kidney cells. The 10th passage of the virus in embryo kidney cells when inoculated on chicken fibroblasts caused a CPE in a few cells, and by the 8th fibroblast passage the CPE appeared extensive. The 8th embryo passage of the Massachusetts strain failed to grow after 5 serial passages in chicken embryo kidney cells.

MATERIALS AND METHODS

The Beaudette embryo-lethal strain 42 and the 398th bird passage of the Massachusetts strain 41, obtained as lyophilized infected tracheal exudate, were used in this study.

Twelve-hour embryo passage of the Beaudette strain of virus was accomplished by inoculating five 10-day-old embryos via the allantoic route using 0.1 ml of undiluted inoculum. The embryos were allowed to incubate precisely 12 hr, at which time 2.0 ml of allantoic fluid was harvested from each live embryo and pooled. A portion of the pooled fluid was inoculated in thioglycollate broth, and another portion was inoculated directly into five 10-day-old embryos. These five embryos were allowed to incubate for 12 hr, and the harvest-inoculation procedure was repeated until at least 7–10 embryo passages were completed. To determine its status, each embryo was candled before inoculation and harvesting. No antibiotics

were added at any time, nor was the material frozen between harvests and inoculations. The harvested fluids were passed undiluted, except in trials 5 and 6, when all inoculums were diluted 1:100 before repassage to determine what effect a diluted inoculum would have upon the final virus concentration. All pools of harvested material were frozen at –60 C after each inoculation was completed, and later titrated by tenfold serial dilution in tryptose broth.

A series of 7 trials were conducted according to the procedure outlined. In addition, one trial was conducted with embryos from IB-susceptible dams to determine whether parental immunity had an effect upon the final virus concentration. One trial was conducted with the Massachusetts strain 41 that had undergone 164 embryo passages. This trial was done to determine whether the effects observed with the Beaudette strain were common to another IB strain of virus.

All titers were compared to the LD_{50} titers obtained by conventionally passing the virus as described by Cunningham.[4]

Twenty-four-hour passage of the Massachusetts strain 41 of virus was performed in much the same manner as the 12-hour passage of the Beaudette strain except that the incubation period was extended to 24 hr, and more embryos were used per passage. The actual virus passage required 4–5 embryos. The remainder of the embryos were allowed to incubate 7 days for observation of the mortality pattern. The virus was harvested at the 24-hr interval and propagated for 300 passages. When interruptions in the virus passages occurred, the harvested material was frozen at –60C until embryonating eggs were available. Every 4th-passage harvest was titrated in embryos, and the EID_{50} and ELD_{50} were calculated. Material from every 20th passage was inoculated into 7 birds via the intratracheal route (100,000 EID_{50} per bird). Inoculated birds as well as two uninoculated control birds were confined singly in Horsfall units. Preinoculation and 4-week postinoculation blood samples were collected from all birds and the sera tested by serum-neutralization (SN) test. Neutralization indices were calculated by the method of Reed and Meunch.[11]

Table 1. Summary of the titers (EID_{50}) obtained by rapid embryo passage of the Beaudette and Massachusetts 41 strains of virus.

Rapid passage no.	Beaudette strain										Mass. 41 164th EP
	Trials with immune embryos							GMT	Susc. embryos	Conv. passage	
	1	2	3	4	5	6	7				
1	8.4	8.5	5.5[1]	8.3	7.6	7.8	6.6	7.87	7.7	7.4	8.4
2	5.6[1]	8.6	5.5[1]	8.6	7.7	8.5	8.5	8.38	8.4	6.2	8.4
3	5.6[1]	8.8	5.5[1]	8.3	8.4	8.2	8.0	8.32	8.0	7.3	8.4
4	7.2	8.8	8.0	9.0	8.3	7.6	8.2	8.19	7.8	7.3	8.6
5	8.6	9.0	8.5	8.5	8.3	8.4	8.5	8.54	8.4	7.5	8.8
6	9.2	8.6	9.2	8.3	9.3	8.4	8.5	8.78	7.6	5.6	8.4
7	8.4	8.2	9.0	8.6	8.2	8.3	8.6	8.50	8.2	7.0	8.3
8	9.3	8.5	8.8	9.0	8.2		8.5	8.71	8.4	6.5	8.0
9	8.6	8.2	9.5		6.5		9.2	8.40	7.3	7.0	
10	8.6	8.8	9.2					8.85	7.8		

[1] Amnionic-fluid harvest titers not included in GMT.

The 1st, 206th, 236th, and 300th virus-passage materials were inoculated into chick embryo fibroblast cultures and examined daily for microscopic evidence of cellular degeneration.

The cell cultures were prepared by trypsinizing 8- to 9-day-old decapitated embryos and suspending the washed cells in tissue-culture (TC) medium 199 (Difco) containing 2% calf serum. The medium was replaced at inoculation time with TC medium 199 without serum. The tissues were harvested after 4–5 days and repassed undiluted until 3–5 passages had been completed. The 1st and 206th embryo passages of the virus were carried through 5 TC passages, and the 236th and 300th embryo passages through 3 TC passages. Embryonating eggs were inoculated with TC harvests to determine the presence and concentration of the virus.

RESULTS

Table 1 summarizes the seven 12-hour rapid embryo-passage trials with the Beaudette 42 virus. The geometric mean titers (GMT) express the results of all trials at each embryo passage.

The GMT of the 2nd rapid passage indicates a threefold increase in titers over the 1st passage. Subsequent passages do not vary significantly. No significant differences are noted between the results of trials 5 and 6, in which the inocula were diluted 1:100, and the results of the other trials. However, the virus titers were greater than those obtained with the virus propagated and harvested at 24–48 hr from dead embryos.

The trial conducted in embryos from IB-susceptible dams shows no significant differences in titers from the trials conducted in embryos from immune dams.

The virus titers obtained by 12-hour embryo passage of the 164th embryo-passage material of the Massachusetts strain 41 of IBV do not vary significantly from passage to passage.

The 24-hour embryo passage of the Massachusetts strain 41 changed in mortality rate after about 30 embryo passages (Table 2). Passage of the virus from 30 through 300 embryo passages did not significantly increase the mortality rate,

Table 2. Summary of serial passages of the Massachusetts strain 41 in embryos.

Pass. no.	No. eggs	Daily mortality following inoculation							
		1	2	3	4	5	6	7	
								Dead	Live
0-10	61	9	4	10	2	6	3	4	23
11-20	44	6	5	7	5	3	3	5	10
21-30	53	6	14	13	4	7	5	3	1
31-40	55	12	18	16	4	1	1	3	0
41-50	39	7	12	11	3	2	0	1	3
51-60	73	20	24	7	8	7	4	1	2
61-70	76	18	23	13	9	9	3	1	0
71-80	81	16	18	22	7	8	1	3	6
81-90	86	25	22	15	7	6	4	5	2
91-100	83	23	21	14	8	9	3	1	4
101-110	76	23	22	17	3	3	5	3	0
111-120	81	22	25	9	10	4	2	2	7
121-130	92	27	19	22	11	3	2	3	5
131-140	110	14	21	31	16	11	8	5	4
141-150	111	19	25	29	6	12	6	3	11
151-160	92	5	30	23	12	8	6	5	3
161-170	128	6	19	32	20	15	17	9	10
171-180	101	7	19	28	15	10	12	9	1
181-190	104	10	26	29	20	8	6	5	0
191-200	113	9	31	28	21	13	4	5	2
201-210	122	13	42	36	12	10	4	3	2
211-220	109	14	34	28	11	13	5	2	2
221-230	108	16	30	31	16	6	3	5	1
231-240	108	18	49	17	8	6	6	4	0
241-250	100	20	44	9	7	7	5	4	4
251-260	111	9	42	27	13	5	4	6	5
261-270	123	15	35	28	11	14	8	4	7
271-280	94	12	35	22	8	1	7	1	8
281-290	82	6	21	12	8	4	8	6	17
291-300	99	5	18	14	5	11	7	9	30

although almost all embryos remaining alive 7 days post-inoculation were markedly stunted and curled. An unexplained decrease in mortality rate was observed in the late embryo passages from the 275th embryo passage through the 300th embryo passage.

The titrations of every 4th passage of virus (Table 3) demonstrate an increase in titer of about one log after 150 embryo passages, possibly indicating further embryo adaptation. The virus was not completely embryo-adapted by the 300th embryo passage, however, as indicated by survival of infected embryos after the 7-day incubation period.

The virulence trials in chickens of every 20th passage

of the Massachusetts strain 41 (Table 4) indicate a loss of the antigenic and pathogenic properties after 100 embryo passages. Trial 5 with the 100th embryo passage of the virus produced a drop in the GMT and erratic production of audible respiratory rales. Material from subsequent passages was erratic in the production of rales until the 240th and all subsequent passages, which failed to produce rales in any of the inoculated birds.

The 200th-embryo-passage virus showed a further loss in immunogenic properties, trial 12. Only 4 of the 7 inoculated birds developed immune neutralizing indices without demonstrating respiratory signs. The three birds that failed to reveal neutralizing antibodies were susceptible to challenge with a more virulent virus. When the test of the 200th embryo passage material was repeated, however, 5 of the 7 inoculated birds developed immune neutralizing indices without demon-

Table 3. Summary of virus titers obtained by serial embryo passage of the Massachusetts 41 strain.

Virus pass.	EID_{50} log_{10}	ELD_{50} log_{10}	Virus pass.	EID_{50} log_{10}	ELD_{50} log_{10}	Virus pass.	EID_{50} log_{10}	ELD_{50} log_{10}
4	7.24	—	104	8.32	8.00	204	8.00	7.78
8	7.83	—	108	8.37	8.17	208	7.78	7.50
12	6.74	—	112	7.68	7.50	212	8.62	8.50
16	7.17	6.67	116	7.50	—	216	8.32	8.32
20	6.62	6.33	120	7.68	7.32	220	8.50	8.33
24	6.63	6.37	124	8.50	8.25	224	8.38	8.12
28	6.83	6.83	128	7.80	7.80	228	7.78	7.62
32	7.20	6.84	132	7.38	7.38	232	8.62	8.00
36	7.74	7.20	136	7.55	7.36	236	8.17	7.82
40	7.88	7.32	140	8.17	7.81	240	7.68	7.50
44	8.17	7.83	144	8.66	8.66	244	8.00	8.00
48	7.83	7.79	148	9.37	9.17	248	7.62	7.62
52	7.38	7.38	152	8.78	8.78	252	8.32	8.00
56	7.37	7.17	156	8.32	8.00	256	8.32	8.32
60	6.00	—	160	8.50	8.50	260	7.62	7.50
64	7.83	7.68	164	8.38	8.25	264	8.50	8.37
68	6.61	6.47	168	8.38	8.17	268	7.83	7.40
72	7.50	7.50	172	8.38	8.38	272	9.38	9.17
76	7.32	7.00	176	8.25	8.25	276	7.83	7.62
80	7.84	7.84	180	8.62	8.62	280	8.38	8.38
84	7.00	7.00	184	8.50	8.50	284	8.50	7.65
88	7.66	7.50	188	8.33	8.33	288	8.50	7.59
92	8.84	8.68	192	8.38	8.38	292	8.40	7.38
96	7.54	7.54	196	8.32	8.16	296	9.50	9.37
100	8.17	7.68	200	8.50	8.50	300	8.17	7.57

Table 4. Pathologic and immunologic response in birds[1] inoculated with the rapid embryo passages of the Massachusetts strain 41.

Trial no.	Virus pass. no.	Post-vaccination results: Pre-vacc. SN (GMT)	SN (GMT) Inoc.	SN (GMT) Controls	Serology[2]	Clinical signs[3]	Challenge[4]
1	20	1.2	4.6	1.1	7/7	7/7	7/7
2	40	1.2	4.1	0.9	7/7	7/7	7/7
3	60	2.9	4.4	1.8	7/7	7/7	7/7
4	80	2.4	4.4	1.7	7/7	7/7	7/7
5	100	1.8	3.2	0.7	7/7	0/7	7/7
6	120	0.7	3.8	—	7/8	5/8	8/8
7	120	2.1	4.0	2.0	7/7	0/7	7/7
8	140	1.4	4.1	0.7	7/7	4/7	7/7
9	160	0.9	3.1	0.9	5/7	2/7	7/7
10	180	1.8	3.4	1.2	7/7	4/7	7/7
11	200	1.5	2.6	1.4	4/7	0/7	4/7
12	200	1.2	2.6	0.3	5/7	0/7	7/7
13	220	1.4	3.2	0.7	4/7	2/7	7/7
14	240	1.9	3.9	2.1	7/7	0/7	7/7
15	260	1.7	1.9	1.0	3/7	0/7	7/7
16	280	1.3	2.0	0.7	3/7	0/7	5/7
17	300	2.1	3.2	1.8	6/6	0/6	6/6

[1] All control birds were susceptible to challenge at the conclusion of the trial.
[2] No. of immune NI per total inoculated.
[3] No. of birds with clinical signs per total inoculated.
[4] No. of birds refractory to challenge per total inoculated.

strating any respiratory signs, and all birds were resistant to challenge.

Trial 17 with the 300th embryo passage revealed an increase in immunological properties, although no increase in pathogenicity was observed. A decrease in embryo lethality had also occurred at the 280th embryo passage (Table 2), suggesting that the virus may have reverted to the characteristics of any early-embryo-passage virus without an increase in host pathogenicity. No explanation can be offered for this change.

All control birds in all trials remained susceptible throughout the trials as determined by serologic and challenge tests. In some trials, the GMT values at the prevaccination bleeding were considered above the susceptible range and above the control birds' GMT 4 weeks later. This may be explained in part by the work of Jungherr and Terrell,[10] who detected passive antibodies to the IBV in 4-week-old chickens. However,

some birds gave high prevaccination titer that cannot be explained by parental immunity, and no explanation can be offered.

In the tissue-culture trials of the Massachusetts strain 41, the 1st embryo passage was lost at the 2nd tissue-culture passage. Recovery of the virus after the 1st passage was probably due to residual initial inoculum, not to virus multiplication. Although the 206th, 236th, and 300th embryo passages of the virus grew in the fibroblast cultures, no cytopathogenicity was observed.

DISCUSSION

The method of rapidly passing the Beaudette strain 42 at the peak of the logarithmic phase of growth appears, on the average, to increase the virus concentration threefold. The threefold increase in virus concentration is apparent after the 1st passage, and further passages do not significantly increase the virus titers. The reason for this phenomenon is not clear. The possibility exists that the harvests of virus at the peak of the log phase of growth may have a higher proportion of live virus particles than a virus harvested at a later time. Since dead virus particles may tie up susceptible cells of the allantoic cavity,[6] a virus inoculum with a higher proportion of live particles may result in a higher virus concentration at harvest time.

Although an increase in virus titer was observed with the Beaudette strain by rapid 12-hour embryo passage, no increase in virus titer was detected when the 164th embryo passage of the Massachusetts strain 41 was employed. The fact that the Massachusetts virus was not completely embryo-adapted may explain why no increase in titer occurred. Hitchner and White[7] demonstrated that nonembryo-adapted strains of IBV are at the peak of their log phase of growth 24–30 hours postinoculation. The 12-hour postinoculation harvest period may have been too short to develop maximum virus concentrations.

Rapid 24-hour embryo passage of the Massachusetts strain 41 of virus in embryos appeared to increase embryo mortality after about 30 passages. Subsequent passages, how-

ever, appeared to affect embryo mortality at a much slower rate, if at all. A total of 300 embryo passages did not cause consistent 100% embryo mortality during the 7-day observation period. In fact, the embryo mortality pattern appeared to stabilize after 100 embryo passages.

The rapid passage of this strain of virus was initially undertaken to try to select the faster-proliferating virus and to pass this virus variant through many embryo passages to build up the variant concentration. It was expected that this faster-growing variant could produce early embryo mortality, and since embryo lethality is usually accompanied by a decrease in bird virulence, this faster-growing variant would rapidly become an attenuated virus. However, during this experiment no evidence of this was observed. Serial embryo passage decreased the invasivensess of the virus as evidenced by a lower incidence of infection of birds inoculated with the late passages of the virus. The immunogenicity of the virus was not lost after 300 embryo passages since some birds were resistant to challenge although respiratory signs were lacking.

Further evidence of embryo adaptation was an increase of about one log in EID_{50} titer in about 150 embryo passages. The 160th embryo passage was also the 1st virus passage tested that failed to produce an SN reaction in all birds tested.

The limited tissue-culture trials have shown that the Massachusetts strain 41 is capable of growing in chick fibroblasts after 206 embryo passages. How many embryo passages are required before the virus will grow in tissue culture was not ascertained. Whether CPE is associated with complete embryo adaptation is not known, since the 300th embryo passage was not completely embryo-adapted.

SUMMARY

Propagation of the Beaudette strain 42 of IBV by harvesting at the peak of the logarithmic phase of growth served to increase the virus titer, on the average threefold, over that of the same virus propagated in a conventional manner.

The increase in titer was observed at the 2nd-passage harvest, and subsequent passages produced no further increase

in titer. An inoculum diluted 100-fold had no effect upon final virus concentration at the 12-hour harvest period. Embryos from IB-susceptible dams used for cultivation of the virus had no effect upon virus concentration when compared to virus harvested from embryonating eggs from IB-immune dams.

Propagation of the Massachusetts strain 41 of IBV by serial passage at the peak of the log phase of growth through 300 embryo passages failed to produce a completely embryo-adapted virus. Some embryo adaptation was observed, as evidenced by an increase in embryo lethality of the virus and an increase in virus titer. The virus revealed a considerable loss in pathogenicity during the late embryo passages, although complete loss of virulence was not observed at the 300th embryo passage. Tissue-culture studies also indicated embryo adaptation of the late embryo passages of virus as revealed by proliferation in chicken embryo fibroblasts.

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ERADICATION OF INFECTIOUS SINUSITIS IN A HATCHERY OPERATION BY SEROLOGICAL TESTING

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ALTHOUGH infectious sinusitis of turkeys has been a major disease problem for many years, no satisfactory method has been devised for control or prevention.

Egg transmission of the infectious sinusitis-chronic respiratory disease agent, a member of the genus *Mycoplasma* (PPLO), was first suggested in turkeys by Jerstad *et al.*,[17] in 1949, and in chickens by Van Roekel *et al.*,[25] in 1952. Since then evidence has accumulated that the infected breeder bird is the primary source of infection.[3,9,11,13,18,20,23,26] Several workers showed that antibiotic treatment of infected breeders reduced or temporarily stopped egg transmission, but infection was not eliminated.[2,21,22,24,27] Thus it is logical that control of infectious sinusitis in turkeys presently depends on eliminating infected breeder flocks from hatching egg-production programs.

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Serological techniques for detecting *Mycoplasma gallisepticum* in fowl were developed by Adler,[1] Jacobs *et al.*[15] and Jungherr *et al.*[19] Adler[1] devised a slide agglutination test for detecting "pleuropneumonia-like" organisms associated with chickens and turkeys. Jacobs *et al.*[15] and Jungherr *et al.*[19] applied the hemagglutination-inhibition (HI) test to chicken serum, a technique soon applied to turkey serum by Fahey.[8] The tube agglutination test was first described by Connecticut workers.[10,16]

Attempts by several workers[2,4,5,6,7,24] to establish Mycoplasma-free commercial chicken breeder flocks by serological means alone or in combination with antibiotic treatment have had variable success.

This report describes the first two years of an infectious sinusitis eradication effort conducted in cooperation with a commercial Texas turkey breeder-hatchery operation.

MATERIALS AND METHODS

The cooperating hatchery had, for years, followed the practice of eliminating all supply flocks with a history or clinical evidence of infectious sinusitis. The cooperative program consisted of serological testing of all supply flocks and elimination of any infected flocks. The birds were tested when over 4 months old, usually at the time blood samples were submitted to the pullorum-typhoid laboratory.

In many instances, 100 preliminary random samples were run 3–6 weeks before pullorum-typhoid testing. This procedure not only avoided the expense of a 100% bleeding for pullorum-typhoid testing if Mycoplasma reactors were found, but also strengthened the degree of accuracy of negative Mycoplasma tests.

During the first test year, 100 random serum samples were tested from each supply flock. This was considered adequate to detect 5% infection at a 99% confidence level. During the second test year, all supply flocks tested were progeny of flocks that had tested negative the previous year. A 100% test was applied to each flock during the second test year.

The serum plate test, using American Scientific Laboratories (ASL) CRD plate antigen and undiluted fresh serum,

was routinely applied to all serum samples during both test years. Reactions up to and including 2+ were considered suspicious, and reactions above 2+ were considered positive. During the last half of the second test year, the HI test, using antigen prepared according to Hofstad *et al.*,[13] was applied to samples that had reacted to the plate test. When 4-unit antigen was used, HI titers up to 1/20 were considered negative, titers of 1/40 were considered suspicious, and titers above 1/40 were considered positive. Flocks were considered free of Mycoplasma when no reactions were observed to the serum plate test or when the HI test indicated that serum plate-test reactions were nonspecific.

Whenever serology was inconclusive, selected birds were examined in the laboratory. Air sacs, and trachea and sinus exudates, if present, were collected as aseptically as possible and suspended in sterile nutrient broth. Mycoplasma isolation attempts were made by inoculating Grumbles' medium[12] and by infraorbital sinus and trachea inoculation of two to four 6 to 20-week-old turkeys that were clinically and serologically negative for infectious sinusitis.

Positive confirmation of breeder flock infection was considered to have been made when: 1) samples yielded Mycoplasma on direct isolation attempts in artificial media, and/or 2) inoculated turkeys developed infectious sinusitis and/or became serologically positive during a 30-day post-inoculation period.

Eggs were not set from any suspect flock until laboratory examinations were completed. In all instances flocks that were serologically positive, and suspect flocks that proved infected, were marketed.

RESULTS

During the two-year period 106 flocks representing 150,540 birds were serologically tested and classified. Of the 106 flocks, 98 were classified negative, and 8 infected (Table 1).

In 1958, 71 flocks representing 100,000 birds were examined by the serum plate test. On initial test, the incidence of reaction was enough to classify 5 of the flocks as positive on

Table 1. Summary of testing results
(January 1, 1958 to December 31, 1959)

	1958	1959	Total
Flocks tested	71	35	106
Birds represented	100,000	50,540	150,540
Birds tested	7,612	41,650[a]	49,262
Flocks positive	8	0	8
Flocks negative	63	35	98

[a] One flock of 9,000 birds was sold for economic reasons before a 100% test. The 110 birds tested in this flock were negative to the serum plate test.

serology alone (Table 2, flocks A, B, C, D, H.) Of 11 flocks classified as suspicious by serology, 3 were determined positive, and 7 negative, by turkey inoculation and/or culture. One flock (M) was termed negative in a second test, and no birds were examined.

Flock F (Table 2) had no clinical symptoms but was classified as infected on the basis of serologic test. The flock, moved from one farm to another on a cool, wet day after being classified, developed clinical symptoms within 10 days of moving.

Flock L (Broad Breasted Bronze) developed clinical symptoms 4 months after testing. This flock had two suspect birds on the first test, and a second negative test on 100 birds 4 weeks later. The two suspect birds were examined and had no lesions. Material was collected from the trachea and air sacs and inoculated into media and the sinus of 3 turkeys, with negative results. Upon investigation a flock of chickens was found on premises within 500 yards of the turkey retaining fence. The caretaker had not noticed chickens in the turkey pen, but contact was assumed to have been possible. The disease was explosive in nature, and almost exclusively of the air-sac form. This flock was eliminated immediately, and all eggs collected were destroyed. No clinical symptoms developed in progeny already hatched. Since these birds were producing commercial-type crosses, none of the progeny were retained as breeders.

In some of the suspect flocks, from 1 to as many as 5 or 6 nodules a few mm in diameter were noted on the abdominal

Table 2. Summary of results[1] on positive and suspected flocks (1958)

	CRD serum plate test					Laboratory findings on reactor birds[6]					
			Results				Serology		Isolations		
Flock	No. birds	No. tested	Pos.	Susp.	Neg.	No. examined	Plate	Tube	Culture	Turkey inoc.	Flock classification
A[2]	3500	100	70	7	23	0					+
B[2]	4000	54	26	2	26	0					+
C	4000	100	41	29	30	0					+
D	1561	100	96	4	0	0					+
E	2800	100	5	10	85	3[5]	+		+	+	+
F[3]	1495	100	2	2	96	2	+		—	+	+
G	1753	100	3	7	90	4[5]	+		—	+	+
H	937	100	9	5	86	4[5]	+		—	+	+
I	542	200	3	0	197	3[7]	—		—	—	—
J	520	100	1	0	99	1[7]	—		—	—	—
K	895	100	1	0	99	1[7]	—		—	—	—
L[3]	515	100	0	2	98	2[7]	—		—	—	—
M[4]	2400	100	1	6	93	0	—		—	—	—
N	2225	100	1	0	99	1[7]	—		—	—	—
O	567	100	0	2	98	2[7]	—		—	—	—
P	1275	100	0	7	93	2[7]	—	—	—	—	—
Q	520	100	0	14	86	2[7]	—	—	—	—	—

[1] +, positive; ±, suspicious; —, negative.
[2] Clinical symptoms at time of test.
[3] Clinical symptoms developed after test.
[4] A retest of 100-bird sample 3 wk later, gave none positive and 1 suspicious.
[5] Air sacculitis discovered at examination.
[6] Blanks indicate no test made.
[7] No gross lesions at necropsy.

air sacs on necropsy. Histopathological examination of some nodules demonstrated a residual fungus infection assumed to be *Aspergillus fumigatus*. In other cases these nodules were broken up and inoculated into turkey sinuses. None of the inoculated turkeys developed signs of sinusitis. When suspect birds were examined closely for these nodules, almost all non-specific reactors showed them, though in some cases they were difficult to find. In every case when blood samples were taken from these birds at the time of examination, they had reverted to the negative state on the serum plate test.

During 1959, 35 flocks representing 50,540 turkeys were examined serologically by the serum plate method. Using a 100% sample technique, 26 flocks were found to be without reactors and were classified negative. Prior to the availability of HI test antigen, six flocks (A-F, Table 3) were suspect on the basis of the serum plate test. Birds from the six flocks, submitted to the laboratory for examination, were determined to be free of infectious sinusitis. Three flocks (G-1, Table 3) that were suspect on the basis of the serum plate test were classified negative by HI test.

Two months following test, one white turkey flock containing 2,747 birds developed clinical infectious sinusitis (lower form). The flock was disposed of after infection was confirmed by serological test, culture, and turkey sinus inoculation. This flock and some of its progeny had the only proved cases of infectious sinusitis associated with the hatching operations in 1959. The sources of the infection could not be determined, but the timing of the outbreak suggested introduction of infection after testing rather than a breakdown in testing procedure.

DISCUSSION

The results offer strong evidence that infectious sinusitis can be eradicated by combining sound management practices with a serological testing program that eliminates breeder flocks containing infected birds.

The serum plate test with ASL-CRD plate antigen proved extremely reliable as a basic screening serological test. "False

Table 3. Summary of results[1] on suspected flocks (1959).

	CRD serum plate test					Other laboratory findings[5]					
			Results				HI test		Isolations		
Flock	No. birds	No. tested	Pos.	Susp.	Neg.	No. examined	+	—	Cult.	Turkey inoc.	Flock classification
A	478	478	1	2	475	2[2]			—	—	—
B	1242	1242	1	9	1232	3[3]			—	—	—
C	1799	1799	4	5	1790	4[4]			—	—	—
D	985	985	0	7	978	3[3]			—	—	—
E	1127	1127	1	7	1119	2[2]			—	—	—
F	1620	1620	1	2	1617	1[2]			—	—	—
G	948	948	1	2	945	0	0	3			—
H	1843	1843	1	2	1840	0	0	3			—
I	2045	2045	1	0	2044	0	0	1			—

[1] +, positive; ±, suspicious; —, negative.
[2] No gross lesions observed.
[3] Air sacculitis in one; no lesions in others.
[4] Aspergillosis in all four.
[5] Blanks indicate no tests made.

positive" reactions were only occasional, and were easily evaluated by applying the HI test to reacting serum.

The 100-bird random sampling technique was reliable in the year it was used, but experience with other diseases (such as pullorum) dictates using a 100% test when possible.

To be acceptable to industry the costs of such a program must be minimal. Eradication was accomplished with minimal losses to the cooperating hatchery. The low initial rate of infection was responsible, a factor in all likelihood resultng from years of diligence in reducing sinusitis losses by not allowing known infected birds to produce hatching eggs and by following sound management and sanitation practices.

Current random sampling of Texas turkeys for serological evidence of infectious sinusitis indicates that about 40% of Texas breeder flocks are infected. This level is high, but appears low enough for large-scale eradication efforts if reintroduction of infection through interstate traffic in breeder replacement eggs and poults or from chickens or other sources can be controlled.

SUMMARY

Eradication of infectious sinusitis in a hatchery operation by the serological testing of breeders is described. During a two-year period 106 flocks representing 150,540 birds were tested. In 1958, 71 flocks representing 100,000 birds were examined, of which 8 flocks were determined to be infected and disposed of. In 1959, 35 flocks representing 50,540 birds (all progeny of 1958 negative-test birds) were examined, and no infected flocks were found.

Two negative supply flocks (one each year) developed infectious sinusitis several months after test. Both outbreaks suggested a post-test introduction of infection rather than a breakdown in testing procedure. Except for the progeny of one of these two flocks, no birds produced under the program developed infectious sinusitis.

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GROSS ABNORMALITIES IN CHICK EMBRYOS DUE TO CONTINUOUS HIGH TEMPERATURE

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INTRODUCTION

EXPERIMENTAL production of malformations in vertebrates has been reviewed by Kalter and Warkany.[12] Recent findings by Orts-Llorca *et al.*[13] and Bergquist,[5] are significant. The former workers ligated the left vitelline vein and obtained microphthalmia and malformations of the left side of the brain; the latter obtained neural overgrowths by surgical

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disturbance of the tip of the notochord and the overlying neuromere.

The present paper is concerned with the effects of temperature on the development of chick embryos. Alsop[1] obtained neural anomalies from both high and low temperatures (109 and 94° F) in chick embryos incubated 72 hours in a noncirculating or still-air incubator. Harman[10] related higher temperatures in this type of incubator to mortality and anomalies, but did not elucidate the types of abnormalities. Ancel and Lallemand,[3] changing the temperature after 48 hr of normal-temperature incubation, obtained high percentages of a single anomaly, celosomia. This condition was obtained in specimens at 39–40, 40–41, and 41–42° C for the fourth through sixth days of incubation. Deuchar[8] obtained notochordal and neural abnormalities in chick embryos with high-temperature shocks durng the first days of incubation.

Barott[4] showed the effects of temperature and humidity on metabolism in the chick embryo. Harrison and Klein[11] showed that a lowered temperature (25° C) at the beginnng of incubation greatly retards the growth of chick embryos. Ancel[2] obtained neural and eye abnormalities in chick embryos from temporary interruptions of incubation. Rott[16] showed that at 38.5° C the growth rate of chick embryos is accelerated in the earlier stages, visceral enlargement is pronounced, and incubation mortality is increased. That writer also observed that, at 36.5°C the earlier stages of growth are somewhat slowed and incubation time is increased.

Patten[14] described malformations of the neural tube in aborted human fetuses of early embryonic age. Brinsmade and Rubsaamen[6,7] obtained neural anomalies in rabbit embryos by injecting pregnant rabbits intramuscularly with cooked milk. Smith[17] obtained neural anomalies in hamster embryos by freezing the mother for a given period during pregnancy.

MATERIALS AND METHODS

The effects of a higher temperature during the entire incubation period were studied. All other conditions were made as constant as possible.

Fertile eggs from a flock of White Leghorn chickens

were collected daily and separated into two groups. A normal-temperature (NT) group was incubated at 37.2–37.8° C, and a high-temperature (HT) group at 39.4–40.0° C. Both incubators were the Humidaire air-circulating rotating-drum type. All eggs were candled daily, beginning on the fifth day of incubation. Infertile eggs were removed when discovered. Dead specimens were removed and preserved in Lavdowsky's fluid. In both temperature groups several live specimens were removed daily from the sixth day of incubation. These specimens were also preserved in Lavdowsky's fluid. All specimens were aged according to the system of Hamburger and Hamilton,[9] weighed, and studied for gross anomalies.

Living specimens previously produced in the NT and HT incubators were studied by serial sections. Routine histological technique, hemotoxylin and eosin stain, and either sagittal or transverse serial sections at 10 μ were used. Two or more specimens for each day of incubation from 4 through 10 were prepared in this way from both the HT and NT groups.

OBSERVATIONS

The NT specimens fitted the limits of the developmental stages described by Hamburger and Hamilton.[9] No anomalies were observed in these specimens. Among the 115 NT eggs, 9 were infertile, 4 died in the shell, and all others were viable when removed from the shell or at hatching.

The HT specimens expressed several different anomalies. Most frequent was celosomia (Table 1). The writers recognize as celosomia any projection of the visceral organs through the body wall. Specifically, celosomia varied from a projection of either the heart, gizzard, liver, or intestine, a projection of any combination of these structures, or a projection of all of these structures through the ventral body wall. Celosomia was preceded by enlargement of the viscera (above) and, to some extent, the lungs and kidneys.

A less frequent anomaly was crooked neck, occurring alone or with celosomia (Table 1). A single case of acrania was found. No neural anomalies were observed among the 16 HT specimens studied by histologic routine.

Table 1. Distribution of celosomia (C) and crooked neck (CN) among dead and living embryos incubated at 39.4–40.0°C.

	Living		Dead		Total	
	No.	%	No.	%	No.	%
C	71	68.93	42	59.16	113	64.94
CN	1	0.97	3	4.23	4	2.30
C & CN	8	7.77	14	19.72	22	12.59
Normal	23	22.33	11	15.49	24	13.79
Total	103		71		174	

Table 2. Distribution of dead specimens for each day of incubation at 39.4–40.0°C.

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
No.	1	0	3	2	3	5	6	4	4	2	3	1	3	13	13	8
%	1.41	0	4.21	2.81	4.21	7.04	8.43	5.62	5.62	2.81	4.21	1.41	4.21	18.38	18.38	11.26

At HT both developmental stage and weight gains were accelerated through 10 days of incubation, after which acceleration declined progressively through 14 days of incubation. Then, both development stage and weight gains were generally retarded. The retardation was greatest among specimens with celosomia. One live specimen at 20 days of incubation weighed only 8.839 g and had reached stage 41 of Hamburger and Hamilton.[9]

Mortality among the total specimens reported here is not available because 103 specimens were removed arbitrarily when they were alive. The 71 specimens that candling determined to be dead are catalogued by day of death in Table 2.

DISCUSSION

The observations on celosomia differ from those of Ancel and Lallemand.[3] The reason might be difference in interpretation of the meaning of the term or the difference in time of applying HT. Those workers applied HT after 48 hr of incubation.

Crooked neck has not been previously recorded as a result of HT, though Pun's review of literature on dwarfism as a lethal factor in chickens mentioned crooked neck as part of the syndrome.

Table 1 compares the distribution of celosomia and crooked neck among live and dead specimens. The effect of these conditions on mortality is not indicated by our work.

Rott[16] also noted accelerated growth in the earlier days of HT incubation. The mortality distribution is highest toward the end of incubation and reaches its next most serious proportions between 11 and 12 days of incubation. The 16 specimens studied by histological section did not show neural anomalies as described by Bergquist,[5] Brinsmade and Rubsaamen,[6] or Orts-Llorca *et al.*[14] Neural anomalies resulting from elevated incubation temperature (Alsop,[1]) were not seen in the above specimens.

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POTENTIATION OF TERRAMYCIN I. EVALUATION OF TEREPHTHALIC ACID AND LOW DIETARY CALCIUM IN BROILER DIETS*

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Interest in the potentiation of tetracycline antibiotics was first stimulated by a report of Peterson.[4] He reported that terephthalic acid (TPA) in poultry feeds increased the antibiotic serum level slightly more than 4 times, and was also associated with a fourfold increase in clinical activity as measured against experimental cecal coccidiosis.

Price and Zolli[5,6] reported that 0.5% TPA in feed increased the serum level of a tetracycline antibiotic two or threefold. They also reported that serum antibiotic level would be similarly increased by reducing the calcium level of the diet to 0.18%. Additional clinical therapeutic activity was therefore suspected. Such was substantiated by Price and Zolli,[5] Cover *et al*.[1] and Peterson,[4] who reported that potentiation of the antibiotic improved benefits in treatment of infectious synovitis and coccidiosis.

More information is needed if potentiated feeds are to be used in broiler production; therefore these experiments were conducted to evaluate further the use of TPA and low-calcium diets for potentiating antibiotics, and to determine the effects of these experimental treatments upon subsequent performance of broilers.

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EXPERIMENTAL

Broiler-type chicks (Vantress males × White Plymouth Rock females) from a commercial hatchery were used. Each bird was vaccinated intraocularly at one day old for infectious bronchitis and Newcastle disease.

The diet, similar to those used in commercial broiler production, contained 1.18% calcium, 0.84% phosphorus, 23% protein, and 1,000 Calories of productive energy per pound. Calcium content was lowered by removing a portion of the ground limestone from the diet. Phosphorus content was also lowered so as to keep the calcium-phosphorus ratio in balance. Adjustments were also made in corn, soybean meal, animal fat content of the diet in order to keep all experimental diets iso-caloric as well as iso-nitrogenous. In all instances the experimental diets were fed for 5 days.

The blood was obtained by heart puncture from a sample of birds from each group. The Terramycin level of the blood serum was determined by the method outlined by Grove and Randall.[3] In the second experiment the serum calcium levels were also measured.

All birds were weighed at the beginning and end of the 5-day potentiated feeding period, and weighed again on the 56th day (end of experiment). Feed conversion was measured for the 5-day potentiation period and for the entire experiment.

Statements of probability are based on analysis of variance according to Snedecor,[7] with significant treatment means determined by the procedure outlined by Duncan.[3]

Experiment 1. Three groups, each consisting of 6 males and 6 females, were fed on each of the 10 experimental diets (Table 1). Birds were raised in battery brooders with raised wire floors. Table 1 shows body weight, feed conversion, and antibiotic blood level. Addition of TPA to the diet containing 1.2% calcium with 210 g Terramycin per ton of feed produced a detectable level of Terramycin in the serum. When calcium level was reduced to 0.5% the antibiotic level in the serum was significantly increased. A further reduction of calcium level to 0.35% did not significantly increase antibiotic level of the

Table 1. Results of feeding broilers various potentiated diets (Expt. 1)

Diet[1]			Body wt. (g)		Gms feed/g gain [3]		
% Ca	Terramycin (g/ton of feed)	Presence or absence of TPA[2]	32-37 day gain	56-day av.	32-37 day	0-56 day	μg Terramycin/ml serum[3,4]
1.2	0	—	193	1399	2.12[a]	2.02	.075[a]
1.2	10	—	203	1459	2.27[bc]	2.04	.075[a]
1.2	210	—	202	1423	2.20[ab]	2.07	.075[a]
1.2	210	+	198	1427	2.23[abc]	2.07	.164[b]
0.5	10	—	203	1453	2.13[a]	1.95	.075[a]
0.5	210	—	186	1458	2.28[abc]	2.06	.229[c]
0.5	210	+	194	1463	2.26[abc]	1.99	.366[d]
0.35	10	—	191	1469	2.42[c]	2.03	.075[a]
0.35	210	—	183	1438	2.37[c]	2.00	.219[c]
0.35	210	+	187	1467	2.11[a]	2.06	.418[d]

[1] These diets fed 32-37 days of age. Basal diet containing 1.2% calcium and 10 grams of Terramycin per ton of diet was fed at all other times except for negative control, which received no Terramycin.

[2] + indicates the addition of 8 pounds of TPA per ton of feed.

[3] Means having different exponential numbers are significantly different according to Duncan's Multiple range test.[5]

[4] Average for determination on 18 birds.

serum. Addtion of TPA to diets containing low levels of calcium significantly increased antibiotic level in the serum. The additive effect of the two methods of potentiation substantiates earlier reports.[4,5,6]

The failure of 0.35% calcium to increase antibiotic level of the serum over that from 0.5% calcium indicates that reducing calcium slightly below that required by the chick gives maximum potentiation of the antibiotic.

Gain in body weight of broilers during potentiation or weight gain during the entire period was not significantly affected by the various experimental feeds. However, the birds on control diets without antibiotics were significantly lighter than the birds that received antibiotics for the entire feeding period. During the potentiation period feed conversion of the broilers receiving the 0.35% calcium was significantly poorer than feed conversion of the birds receiving 0.5 and 1.2% calcium. The difference, however, was overcome by the end of the experiment.

Experiment 2. A second battery experiment was conducted to determine the effect of feeding diets containing graded levels of calcium upon the potentiation of Terramycin.

Table 2. Results of feeding broilers diets containing various levels of calcium during a five day potentiation period (Expt. 2)

% Ca[1]	Body wt. (g)[2] 27-32 day gain	Body wt. (g)[2] 32-56 day gain	µg Terramycin/ml serum[2]	Blood Ca (mg %)
1.2	100[cd]	790	.126[cd]	7.75
1.0	102[bc]	835	.124[d]	6.50
0.8	93[cd]	832	.194[bc]	8.40
0.7	84[f]	815	.219[b]	9.65
0.6	95[cd]	853	.215[b]	7.45
0.5	102[bc]	841	.211[bc]	8.90
0.35	88[ef]	829	.232[b]	6.85

[1] These diets fed 27-32 days of age. Basal diet containing 1.2% calcium fed at other times.

[2] Means having different exponential numbers are significantly different according to Duncan's Multiple range test.[2]

Four replicates of 2 males and 2 females were used per treatment. The results shown in Table 2 were not affected by reducing dietary calcium from 1.2 to 1%. The antibiotic content of the serum was significantly increased when the calcium level was lowered to 0.8%, and a further increase was obtained by lowering the calcium to 0.7%. No further improvement was obtained by reducing the calcium level. This confirms results in the first experiment. Again feeding the potentiated diets for a 5-day period did not significantly affect growth rate or feed efficiency of broilers.

Blood calcium determinations revealed no correlation between blood calcium levels and antibiotic content of the blood serum.

Experiment 3. Birds were grown in floor pens in order to test the use of potentiated feeds with birds grown nearly as possible to practical conditions. A commercial coccidiostat (Amprolium) was used. They were brooded under infrared bulbs, on a litter of wood shavings. Two groups, each containing 38 males and 38 females, were fed each of the experimental diets (Table 3). Potentiated feeds for five days (from 28 to 32 days old) did not affect body weight or feed conversion.

SUMMARY AND CONCLUSION

Terephthalic acid and low-calcium diets were studied to determine their ability to potentiate Terramycin, and to study the effects of feeding these diets upon subsequent growth and

Table 3. Body weight and feed conversion of broilers fed diets containing various levels of calcium (Expt. 3).

% Ca[1]	Body wt. (g)		g feed/g gain	
	28-32 day gain	56-day av.	28-32 day	32-56 day
1.20	126	1252	2.06	2.36
1.00	132	1243	2.04	2.40
0.90	128	1233	2.07	2.38
0.85	135	1250	2.01	2.41
0.80	136	1252	1.99	2.43
0.75	129	1242	2.06	2.36
0.70	131	1227	2.09	2.39
0.60	137	1253	1.98	2.37
0.50	137	1238	1.98	2.53
0.35	129	1249	2.13	2.56

[1] These diets fed 28–32 days of age. Basal diet containing 1.2% calcium fed at other times.

feed conversion of broilers. Both treatments increased the Terramycin content of the serum. Decreasing calcium much below the level required by the chick gave no additional benefits as measured by increased antibiotic content of the serum. Growth rate or feed efficiency during the potentiation of 5 days was not greatly affected by the use of the potentiated diets. Any small depression was overcome by the end of the experiment (56 days old). These experiments indicate that low-calcium feeds may be used for short periods to increase the effectiveness of Terramycin without harming the birds.

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THE EFFECTIVENESS OF SEVERAL TETRACYCLINES IN THE CONTROL OF AN EXPERIMENTAL PPLO (*MYCOPLASMA GALLISEPTICUM*) INFECTION IN CHICKENS

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INTRODUCTION

Early work of Nelson,[6,7] later substantiated by Delaplane and Stuart,[1] strongly indicated an etiological role for pleuropneumonia-like organisms (PPLO) (*Mycoplasma* sp) in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Smibert *et al.*,[8] in 1959, were able to produce all symptoms of air-sac disease by inoculating PPLO into germfree chickens and turkeys. No evidence of viral contamination could be demonstrated in either the inoculum or the lesion tissue of the infected birds. Subramanyam and Pomeroy[9] indicated that the Fahey-Crawley virus[3] only serves to activate a latent PPLO infection. Other agents, such as Gram-negative bacteria, the ornithosis organism and infectious bronchitis virus, have been shown to activate chronic respira-

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tory disease in chickens and infectious sinusitis of turkeys, but it seems well substantiated that the pleuropneumonia-like organism is the primary cause of the two diseases.[9] Hearn *et al.*[4] eliminated a strain of PPLO of nonavian origin from tissue culture with chlortetracycline.

Over the last 4 years a reproducible PPLO infection in chicks[5] has been established that we believe can be used to compare the activity of antibiotics in the treatment of chronic respiratory disease of chickens and infectious sinusitis of turkeys.

MATERIALS AND METHODS

After one-day-old Hall-cross sex-linked cockerels, weighing 36 g, were randomized into groups of equivalent weight, they were infected by inoculation into the yolk sacs with 0.5 ml of a yolk-sac suspension of *Mycoplasma gallisepticum*[2] diluted 10^{-1} in brain heart infusion broth (Difco). The suspension was prepared from embryos dying on the seventh day after infection. This strain of PPLO, obtained originally from Dr. Erwin Jungherr, University of Connecticut, has been passed by yolk-sac through chick embryos for more than 4 years. In each experiment one group of chicks was injected in the same manner with yolk-sac suspensions from uninfected embryos to serve as an internal standard. To apply stress, brooder heat was turned off on the fifth day after infection.

Each test group was 20 chicks. All medication was administered in the diet, which was a simulated commercial broiler mash. All tests were terminated on the 27th day, and at least 60 chickens (3 trials) were used in evaluatng each level of antibiotic.

There were 3 criteria of activity: 1) mortality, 2) mean weight of survivors, and 3) weight (yield) of groups at 27 days. PPLO infection causes a chronic disease, producing death only when stress is severe. Mortality was erratic since stress factors varied because of variations in delivery time. At times nearly all of the infected untreated birds died, and at other times birds weighing less than 100 g continued to live throughout the 27 days of test. The mean weight of surviving

Table 1. Summary of data at 4 wk. old on PPLO infection in birds receiving two levels of antibiotics in feed

Treatment	Survivors	Av. wt. (g)	Av. yield[a] (g)
Normal controls	97% (116/120)	361	7056
Infected Controls	48% (57/120)	316	3037
400 ppm			
CTC	97% (58/60)	365	7066
OTC	88% (53/60)	313	5581
DMCTC	93% (56/60)	327	6109
TC	92% (55/60)	351	5493
200 ppm			
CTC	88% (106/120)	317	5614
OTC	60% (72/120)	306	3758
DMCTC	79% (95/120)	313	4985
TC	65% (39/60)	303	4186

[a] Per group of 20 birds started on treatment.

birds was unreliable because there was less competition for food if only a few birds survived. The most consistent criterion was the total weight of survivors expressed as yield. This value is a function of both the number of survivors and the weight of the surviving birds.

Serum samples taken from the birds at the end of the experiment were tested for agglutinins by the plate technique, using stained PPLO antigen purchased from the Connecticut Agricultural Expriment Station. A large percentage of positive sera from infected groups, especially those with abnormally low weight yield, suggested a causal relationship between degree of PPLO infection and decreased weight gain.

Lesion score was not used as criterion of effectiveness of the drugs since birds which were challenged with only the primary agent of C.R.D. seldom showed pathology greater than a cloudy pericardial and air-sac membranes even though poor growth and agglutination studies showed they were infected with *Mycoplasma gallisepticum*. Cheesy exudative lesions could be produced consistently under our experimental conditions only if infected birds were given a secondary challenge of *E. coli*.

RESULTS

Preliminary experiments had demonstrated that infected birds receiving 50 and 100 ppm of the tetracyclines showed no

Table 2. Statistical summary of PPLO infection at 4 wk. old in birds receiving two levels of antibiotics in feed

Treatment	Av. yield* (g)	Standard deviation	"t" value	Significance
Normal controls	7056	340	5.5	0.1%
Infected controls	3037	1758	—	
400 ppm				
CTC	7066	472	3.7	1%
OTC	5581	1348	2.1	ns
DMCTC	6109	768	2.8	5%
TC	5493	1092	1.7	ns
200 ppm				
CTC	5614	446	2.9	5%
OTC	3758	1502	0.8	ns
DMCTC	4985	1050	2.5	5%
TC	4186	1400	1.6	ns

* Per group of 20 birds placed on test.

significant response. Table 1 gives the per cent survival, mean weight, and yield (per 20 birds placed on test) of infected birds receiving 200 and 400 ppm of four tetracyclines. Per cent survival and mean weight were indicative of the antibiotic response, but yield, which combines these two factors, was more consistent than either. The mean survival time of the birds that died during the test is not reported, but the data show it was longer in the medicated groups (19-26 days).

The yields with the standard deviations and t-value are given in Table 2. Oxytetracycline (OTC) and tetracycline (TC) at 200 and 400 ppm were ineffective at a probability of 1 to 20. At the same levels of medication both chlortetracycline (CTC) and 6-demethylchlortetracycline (DMCTC) gave

Table 3. Statistical comparison at 4 wk. old of chicks treated with chlortetracycline (400 pm) with untreated uninfected controls

No. of chicks	Mean weight	Standard deviation	"t" values*
Experiment 1			
20 untreated	360	29	—
20 treated	378	39	1.6
Experiment 2			
20 untreated	368	29	—
20 treated	349	30	2.03
Experiment 3			
20 untreated	344	46	—
20 treated	368	59	1.5

* t values for 38 degrees of freedom at probability of 5% = 2.02.

Table 4. Effect of chlortetracycline on weight at 4 wk. old of uninfected chicks (FL40).

Conc. in feed	Mean wt.*	Standard deviation	"t" value**
None	360	38.2	—
400 ppm	365	42.7	0.390
200 ppm	355	39.5	0.406
100 ppm	356	41.2	0.315

* Per group of 20 placed on test.
** Significance for 38 degrees of freedom at probability of 5% = 2.02.

a significant response. The t-value for 200 ppm of CTC, however, was about equal to the t-value for 400 ppm of DMCTC, which indicates that CTC was twice as active on a weight basis.

Since the mean weight was slightly higher in birds on 400 ppm than in uninfected birds, a statistical analysis was made to compare uninfected controls with infected birds receiving 400 ppm of CTC. The comparisons are in Table 3. In two experiments the birds receiving 400 ppm of CTC had higher group weights than the uninfected controls, but the difference was not significant. In each case the standard deviation of the weights was greater for the antibiotic-treated birds than for the controls, indicating that the treated birds, though heavier, were not as uniform in size. In the other experiment (No. 2), mean weight was lower for CTC-treated birds than for uninfected controls. The difference was significant at a probability of 5%.

Since, with the data given, untreated uninfected birds cannot properly be compared with treated infected birds, an experiment was run to determine the growth effect of CTC on uninfected birds (Table 4). These data show that, with our high-quality chicks kept under brooder conditions, CTC gave no significant response. Thus, the conclusions drawn from Table 3 should be valid.

Table 5 compares agglutination responses with weights of the birds. Though all survivors of a group were blood-tested, not all groups came from the same experiment. These tests were done when the agglutination procedure was being

initiated, and time and personnel allowed the testing of only one group of birds in an experiment. However, all agglutination tests were done under identical conditions and should be comparable. None of the uninfected birds showed agglutinins. All but 2 of the 8 survivors in the infected untreated group gave a positive response to the antigen. A possible explanation might be parental immunity, which is discussed later.

In the infected medicated groups, agglutinins were present in all birds sick enough to show abnormally low weight gains. However, 4 birds (1 in CTC-treated and 3 in DMCTC-treated groups) had PPLO agglutinins even though growth was normal and gross morphology of birds was not essentially different from that of the controls.

At 4 weeks of age, heavily infected birds (as determined by weight loss and agglutination) showed no exudate in the nostrils, rattling, or gross lesions of the air-sac or pericardial membrane.[6] The pericardial and air-sac membranes of the

Table 5. Weights (g) and agglutinin reaction at 4 wk. old of birds untreated and birds receiving in feed 400 ppm chlortetracycline (CTC) and 6-demethylchlortetracycline (DMCTC)

Uninfected untreated		Infected untreated		CTC		DMTC	
Wt.	Reaction	wt.	Reaction	wt.	Reaction	wt. gm.	Reaction
299	—	110	+	244	—	102	+
322	—	146	+	320	—	120	+
349	—	226	+	330	+	146	+
350	—	290	+	342	—	179	+
331	—	295	+	346	—	270	+
368	—	329	—	352	—	305	—
373	—	356	+	354	—	306	—
377	—	371	—	364	—	306	—
382	—			369	—	318	—
382	—			375	—	324	—
383	—			375	—	326	—
383	—			384	—	330	—
386	—			385	—	344	+
392	—			393	—	345	+
393	—			395	—	348	—
404	—			398	—	350	+
415	—			405	—	365	—
440	—			418	—	370	—
Ex # FL 19		Ex # FL 19		Ex # FL 17		Ex # FL 13	

infected birds were cloudy. Occasionally a cheesy exudate lesion appeared in pockets along the membranes, but the symptoms could not be correlated with weight or mortality data. Heavily infected birds seemed to have less blood and a lower percentage of plasma. Apparently, infecting birds by the yolk-sac route does not give typical CRD symptoms under the conditions of our tests.

DISCUSSION

It should be noted here that the per cent of infection was high, but never 100%. This can be explained, possibly, by work of Kiser *et al.*,[3] which showed that chickens from the same flock gave a positive PPLO agglutinin reaction (7 per 24 and 6 per 24) at 1 day of age. Antibodies transferred from the dam may well be sufficient in some birds to protect them from infection.

The experiments reported above were designed to evaluate the effectiveness of several tetracyclines in suppressing a laboratory *Mycoplasma gallisepticum* infection of chicks. They were not designed to study the more complex field disease known as CRD. Ample references have been cited, however, to substantiate the belief that PPLO organisms are the primary invaders in field cases of CRD. If PPLO organisms can be suppressed or eliminated, clinical symptoms will probably not develop. If clinical lesions have become established, however, it is not implied that the antibiotics at concentrations stated in this paper will clear the lesions of secondary invaders.

The data collected in this investigation can be valuable to the poultryman as indicating a prophylactic measure against CRD, but no claims can be made as to effectiveness in regressing clinical lesions and thus preventing condemnation in a diseased flock.

CONCLUSIONS

The following conclusions appear warranted:

1) On a weight basis, CTC was twice as effective as DMCTC against an experimental PPLO infection in chicks.

2) TC and OTC were not significantly effective at 400 ppm in the feed.

3) At 200 ppm, birds treated with CTC and DMCTC made significantly better weight gains than the infected untreated controls.

4) Infected birds treated with 400 ppm of CTC averaged as heavy as uninfected birds, but were not as uniform in size, as demonstrated by a higher standard deviation.

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VIABILITY OF POX AND LARYNGOTRACHEITIS VACCINES

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LABORATORIES licensed by the U. S. Department of Agriculture to produce vaccines are required to keep samples of each serial lot of each product manufactured. At the Vineland Poultry Laboratories, random samples of each serial lot of vaccine are taken at the time of final packaging of the material. Such samples are held at about 40° F. Many serial lots of various products date back to 1931. This valuable reservoir for comparison with the viruses used in present-day vaccines provided an opportunity to check viability and present protection against disease. Fowl pox vaccine and pigeon pox vaccine have been retained since 1931; laryngotracheitis vaccine samples have been retained since 1934. Beaudette *et al.*[1] reported that a strain of fowl pox virus passed in chicken eggs was active after 3,598 days in the dried state. Pigeon pox virus passed in chicken eggs was active after 3,605 days in the dried state. Laryngotracheitis virus was active after 3,359 days in the dried state.

Since many of our samples were active after very long periods in a dried state, it was thought that this information might be of value to others.

VIRUSES TESTED

Fowl pox. Fowl pox vaccine was originally produced by scarifying the combs of young White Leghorn cockerels with active virus, and scraping 10 days later. The scabs were allowed to air dry, which took up to 72 hours, ground in a mortar wth a pestle, and screened through a 60-mesh screen. The ground material was weighed and placed in a bottle or

Table 1. Viability of Eleven Lots of Fowl Pox Vaccine.

Production date	Time held until test		Take reading	Susceptible birds on challenge
	Yrs. & Mos.			
Paraffin-sealed comb scabs (lots 1–4)				
9/15/31	28	10	0/5	5/5
5/25/35	25	1	0/5	5/5
12/22/36	23	1	0/5	5/5
6/12/38	22	1	0/5	5/5
Vacuum-sealed embryo material (lots 5–11)				
3/ 9/39	21	4	5/5	0/5
9/23/39	20	9	5/5	0/5
10/ 6/39	20	8	5/5	0/5
10/20/39	20	8	5/5	0/5
3/24/54	6	9	5/5	0/5
12/24/54	5	5	5/5	0/5
2/ 3/56	4	5	5/5	0/5
Controls			0/5	5/5

shell vial, which was sealed with a sleeve rubber stopper or a standard cork, which was then dipped into paraffin. The dried material was reconstituted in a glycerine-saline solution for application to birds (lots 1–4, Table 1). From 1939 on, fowl pox vaccine has been produced in embryonating eggs. Eggs are inoculated on the 11th day of incubation and harvested on the 16th day. At that time the chorioallantoic membranes are frozen, placed in a desiccator over phosphoric anhydride, and dried under vacuum from the frozen state. The dried membranes are ground, sieved, weighed, and put into individual ampoules, which are sealed under vacuum (lots 5-11, Table 1). The spark method indicated that vacuum was still present at end of the storage period.

All sample lots, mixed just before used, were applied with a two-needle stick method applicator in the web of the wing. The birds were examined for "takes" in the web of the wing on the 7th, 10th, 12th, and 15th days after vaccination. They were challenged 28 days after vaccination. Examinations for pox lesions were made on the 7th, 12th, and 17th days after challenge.

Laryngotracheitis. Laryngotracheitis vaccine was first produced by injecting active virus material into the sinuses and tracheae of susceptible growing birds. Five days after injection, the birds were killed by the air-embolus method

Table 2. Viability of 21 lots of laryngotracheitis vaccine.

Production date	Time held until test			Take reading	Susceptible birds on challenge
	Yrs. & Mos.				
Chicken exudate, vacuum-dried (lots 1-7)					
11/11/34	25	6	vacuum-packed in ampoules	2/5	3/5
12/25/35	24	6		5/5	0/5
11/19/36	23	7		4/5	0/5
2/11/37	23	5		0/5	4/5
3/30/37	23	3		5/5	0/5
6/22/37	23	—		5/5	0/5
10/6/38	21	9		5/5	0/5
Dried embryo material (lots 8-21)					
1/24/39	21	5		5/5	0/5
3/28/39	21	3		5/5	0/5
7/25/39	20	11		5 5	0/5
11/29/40	19	7		2/5	3/5
8/8/42	17	11		5/5	0/5
9/30/43	16	9		5/5	0/5
10/22/43	16	8		5/5	0/5
9/15/45	14	9		5/5	0/5
3/19/48	12	3		5/5	0/5
11/10/49	10	8		5/5	0/5
1/12/50	10	5		5/5	0/5
1/8/53	7	6	vacuum-stopper packed	5/5	0/5
9/30/54	5	9		5/5	0/5
7/13/56	3	11		5/5	0/5
Controls				0/5	5/5

and the blood and mucous were collected from the sinuses and tracheae. The collected material was immediately frozen, placed in a desiccator over phosphoric anhydride, and dried under vacuum from the frozen states (lots 1–7, Table 2). From 1939 on, laryngotracheitis vaccine has been produced in embryonatng eggs, inoculation being done on the 10th or 11th day of incubation and harvested on the 16th or 17th day. Then the chorio-allantoic membranes are frozen and lyophilized. The ground dried material was reconstituted with a glycerine-saline solution for application to birds (lots 8–21, Table 2).

Both the material from live birds and from embryonating eggs was dried, ground, sieved through a 60-mesh screen, weighed, and put into individual ampoules, which were sealed under vacuum. Since 1953 this vaccine has been placed in small

Table 3. Viability of eight lots of pigeon pox vaccine.

Production date	Time held until test			Take reading	Susceptible birds on challenge
	Yrs. & Mos.				
Paraffin-sealed pigeon scabs (lots 1 and 2)					
4/28/31	29	2		0/5	5/5
9/23/31	28	9		0/5	5/5
Dried embryo material (lots 3–7)					
10/16/39	20	9	sealed-ampoule packed	5/5	0/5
4/12/46	14	3	sealed-ampoule packed	5/5	0/5
7/ 8/51	9	—	sealed-ampoule packed	5/5	0/5
6/11/54	6	—	sealed-ampoule packed	5/5	0/5
5/ 6/55	5	2	sealed-ampoule packed	5/5	0/5
11/11/55	4	8	vacuum-stopper packed	5/5	0/5
Controls				0/5	5/5

glass bottles and sealed under vacuum, specially prepared rubber stoppers being used to achieve this purpose.

Lots 1–18 were packaged in ampoules under vacuum. Lots 19–21 were packaged in bottles with vacuum stoppers. The electric spark method showed the presence of vacuum in all of the ampoules and bottles. All sample lots, mixed just before use, were applied with a brush to the vent of the birds. Birds were examined for "takes" 4 and 5 days later. They were challenged by the intrasinus method with a fully virulent strain of virus 12 days after vaccination.

Pigeon pox. Pigeon pox vaccine was originally produced by applying active virus material to the feather follicles of the breast of a pigeon after the feathers had been removed. Ten days later the scabs were scraped free and allowed to air dry, which took up to 72 hours. The scabs were then ground with mortar and pestle, sieved through a 60-mesh screen, weighed, and placed in a shell vial, which was sealed with a standard cork dipped in paraffin. The dried material was reconstituted in a glycerine-saline solution for application to pigeons or chickens (lots 1 and 2, Table 3).

From 1939 on, pigeon pox vaccine has been produced in embryonating eggs. Eggs are inoculated on the 11th day of incubation and harvested on 17th day. At that time the chorioallantoic membranes are frozen, placed in a desiccator over phosphoric anhydride, and dried under vacuum from the frozen state. The dried membranes are ground, sieved, weighed, and put into individual ampoules, which are sealed under vacuum (lots 3–7, Table 3). Since 1955 the dried material has been placed in a bottle under vacuum, a special rubber stopper being used to achieve this purpose (lot 8, Table 3).

The electric spark method showed the presence of vacuum in the ampoules and bottles in lots 3–8. All sample lots, mixed just before use, were applied with a brush to 20 feather follicles on the outer surface of the leg. Birds were examined for takes on the 7th, 10th, 12th and 15th days after vaccination. Challenge was 28 days after vaccination. Examinations for pox lesions were made 7, 12, and 17 days after challenge.

SUMMARY

Fowl pox vaccine held 21 years and stored at 40°F protected birds against challenge doses of fowl pox virus. Only vaccines sealed under vacuum retained ability to immunize. Four lots of fowl pox vaccine held from 22 to 28 years, made from scab material from live birds and packaged in bottles or vials sealed with either rubber stoppers or corks were inactive.

Laryngotracheitis vaccine held 25 years at 40°F immunized 40% of the birds. Vaccine held 24 years protected 100%.

Pigeon pox vaccine held 20 years at 40°F protected all birds. Two lots of vaccine produced on pigeons, held 28 and 29 years, packed in shell vials and sealed with corks dipped in paraffin, were inactive.

Preservation of dried virus-containing material under vacuum effectively maintains the viability of fowl pox, pigeon pox, and laryngotracheitis vaccines.

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SPIRAMYCIN IN THE TREATMENT OF INFECTIOUS SINUSITIS IN TURKEYS

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SPIRAMYCIN, a new antibiotic,[10,11] has an antibacterial activity similar to erythromycin. Hamdy *et al.*,[3,4] Holper *et al.*,[5] and Barnes *et al.*[1] have reported on the use of erythromycin in the treatment of turkey sinusitis. The action of Spiramycin against experimental Mycoplasma infection has been reported.[6] Pleuropneumonia-like organisms (PPLO) have been found to be the cause of turkey sinusitis, both experimentally[2,3,5,7,8] and in naturally infected birds.[2,3,7] Infectious sinusitis is a serious problem in many turkey flocks, and new and better treatments are constantly being sought. Since Spiramycin had been reported effective in controlling PPLO infections,[6] its use was investigated for treating infectious sinusitis in experimental and field cases.

MATERIALS AND METHODS

Spiramycin adipate powder was dissolved in sterile distilled water for injection or mixed in the feed immediately before use. No fluid was removed from any sinus prior to treatment.

Turkeys, 4 to 8 weeks of age, were injected in the left sinus with 1 ml of S6 strain of PPLO culture obtained from Dr. H. E. Adler, University of California. Either phenol-red broth cultures or egg-yolk material was used and the infected birds were held in isolation until enlarged sinuses developed.

For field trials, flocks were selected in which sinusitis was a serious problem. In two instances (flocks A and C), infected turkeys with large swollen sinuses were penned separ-

ately from the main flock for treatment; in a third trial (flock B), the entire flock was treated. Degree of recovery was judged by clinical evidence of sinusitis. Recovery was recorded only if all swelling and fluid had disappeared from the sinus.

No effort was made to isolate PPLO from the field cases, and no serological studies were made. In 10 experimental cases, sinus swabs and tracheal sections were cultured for PPLO after recovery. The experimental cases were indistinguishable, clinically and morphologically, from the natural cases in commercial flocks on range.

RESULTS

Experimental infection. Three of 4 sinuses treated with 100 mg of Spiramycin recovered. Ten of 11 sinuses injected with 50 mg recovered and 8 of 9 injected with 25 mg recovered.

A pen of 7 turkeys, all with large swollen left sinuses, were given medicated feed containing 50 g of Spiramycin per ton for 19 days. Five days after treatment, one sinus remained swollen, and it was reduced to about half size.

A third group (28 turkeys), some with severe bilateral sinusitis, was fed Spiramycin at 200 g per ton of feed for 9 days. Twenty-two days after treatment, 10 turkeys still had varying degrees of sinusitis in one or both sinuses and a few remained large and tightly swollen.

Swabs from the left sinuses and small pieces of the tracheas from 10 experimentally infected turkeys were cultured in phenol-red broth and on Morton's media. Five of these had been injected in the left sinus with 100 mg of drug and recovered, and the other 5 had recovered after being fed 50 mg per ton for 10 days. Viable PPLO were recovered from all 10 turkeys. At necropsy, 2 had air sac lesions though recovery was clinically complete.

Field studies. Flock A. From a range flock, 100 turkeys with one or both sinuses affected were penned separately and fed 100 g Spiramycin per ton of feed for 10 days. One week later, 72 turkeys still had swollen sinuses. Infected turkeys left in the flock as controls had not recovered. At this time,

each affected sinus from the treated group was injected with 100 mg of Spiramycin. Fourteen days later, 68 turkeys had recovered, leaving 4 with some swelling. Then, 61 of the controls were injected with 100 mg per sinus and the 4 from the previous group were treated again. Fourteen days later, all of the 61 were clinically recovered except 3 turkeys each with both sinuses markedly swollen and turgid. The 4 that were retreated recovered after the second injection.

Flock B. A range flock of 850 turkeys, 12 weeks of age, showed a respiratory infection characterized by sneezing, wiping the nostrils on the wings, and nasal exudate. One or both sinuses of 41 were swollen. The entire flock received 120 g of Spiramycin per ton of feed for 16 days. Five turkeys with respiratory infection and sinusitis were held in a separate pen as controls. At the end of medication, all the clinical respiratory signs had disappeared and nearly all sinus swelling had receded. The controls continued to show respiratory signs and sinusitis.

Flock C. Eighty turkeys which had shown clinical signs of sinusitis for 10-12 weeks were selected from a flock of 1,000 birds, 20 weeks old. They were fed 200 g of Spiramycin per ton of feed for 9 days. Twenty-one days later, 35 had recovered, 37 were improved, and 8 had not changed.

DISCUSSION

Spiramycin caused clinical recovery from infectious sinusitis when injected directly into sinuses in 100-mg doses. Smaller doses were similarly effective but the number of treated sinuses was much smaller. The drug was less effective and results were more sporadic when it was mixed in the feed even up to 200 g per ton with the one exception in which 6 of 7 turkeys recovered when fed 50 g per ton.

Even though birds appeared to recover after injection of 100 mg into the sinus or from eating feed containing 50 g per ton, PPLO were recovered from 10 turkeys by culturing sinus swabs or small pieces of tracheas in phenol-red broth or Morton's media.

Kiser *et al.*[5] treated chickens experimentally infected with PPLO and reported favorable results at levels of 100,

200, and 400 ppm in the feed. His results are not directly comparable to those reported here, however, because he started treatment the day of infection or the day following. In all of these trials, the birds had been infected 30 days or longer before being treated.

The effectiveness of Spiramycin in the treatment of sinusitis when injected into the sinus would seem to justify further investigation of this drug against PPLO infections.

SUMMARY

Experimental and natural cases of turkey sinusitis were treated wtih Spiramycin, a new antibiotic. The drug was effective when injected into the sinuses but less effective when given in the feed.

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GENETIC DIFFERENCES IN RESISTANCE TO NEWCASTLE DISEASE

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DEMONSTRATIONS of genetic susceptibility or resistance to specific disease-producing agents aid in focusing attention upon the important role that genetic constitution of a stock plays in its over-all performance. Differences in resistance to Newcastle disease are to be expected, but no clear-cut evidence has yet been published. This report presents evidence that such differences do exist between strains and among sire families within a susceptible strain.

REVIEW OF LITERATURE

Godfrey[2] suggested that differences within several stocks of birds in duration of a pause in egg production after an outbreak of Newcastle disease indicated differences in susceptibility. However, in the groups compared there was no relation between rate of mortality from this disease and the length of pause for the survivors. For example, in Barred Rocks mortality was 2.6% and average pause 33 days, whereas in White Rocks mortality was 6.2% but pause only 17.3 days.

Francis and Kish[1] attempted to select for resistance to intramuscular inoculation of Newcastle-disease virus. Their statistical analyses indicated some differences between families in duration of survival after such inoculations, but the poor viability of their Family D, even when not inoculated, could have contributed more to the "t-values" than differences in resistance to Newcastle virus *per se*. Continuation of the project was hampered by extremely high mortality (92.7%) following vaccination, which obscured any genetic differences.

The reports of Iyer and Dobson[4] and Teklinski[5] do not provide conclusive evidence of genetic differences in resistance

to Newcastle disease. Their observations were probably confounded by egg-borne passive immunity, the existence of which for this disease was unknown to them at that time.

HISTORY OF STOCKS COMPARED

Following a mild outbreak of Newcastle disease among adults in the late winter of 1947–48, it was our practice until recently to vaccinate routinely all young stock at 10–16 weeks of age, using wing-web vaccine of commercial origin.

In 1955, for the first time, the vaccine produced a prompt and marked effect upon the birds. Mortality as a direct consequence of the vaccination was high enough to cause some concern, and morbidity was rather general.

In 1956 the vaccination procedure caused an even more severe reaction than in 1955. Mortality due to vaccination was higher than in the previous year. Other stocks of pullets, including those of the two New York Random Sample Tests and that of the Cornell Random-bred population, which had been given intraocular Newcastle vaccine (mild B_1 strain) at four weeks of age did not show any adverse response to the wing-web vaccine. Diagnosis of Newcastle disease was based (as in 1955) upon the prevalent symptoms, the absence of other indications of disease, and the fact that the losses occurred shortly after administration of the live-virus vaccine, well within the normal range of its incubation period. Many of the birds showed the typical nervous symptoms, the complete prostration, the blurred eyes and early death. In a few that did not succumb immediately, permanent paralysis of one or more limbs developed.

Each year the birds under study had been exposed in late May to a virulent strain of infectious bronchitis virus, administered intraocularly or intratracheally to two birds in each group of 125. Four weeks later the Newcastle vaccine was given to all birds on one morning. No drugs or antibiotics were given, and no birds were culled except those which were in the final stages of some disease or unable to get to feed and water. Paralyzed birds were transferred from the rearing range to colony houses where feed and water were more accessible and where they would not be molested by other

birds or killed by predatory animals. Those failing to show some degree of recovery in a reasonable time were destroyed and examined. A few birds recovered, except for a permanent loss of control over a leg or wing. Ten such pullets were eventually autopsied and included as mortality due to Newcastle disease.

MORTALITY FOLLOWING VACCINATION

Strain diferences. In 1955, during the fourth day after vaccination, presence of the disease was obvious as indicated by paralysis and other symptoms of involvement of the nervous system. A few birds died on the 4th and 5th days. During the 5th and 6th days many more birds developed clinical symptoms and were removed, because of paralysis, to colony houses. A number of these became unable to eat or drink and were therefore destroyed on the 11th or 12th day after vaccination.

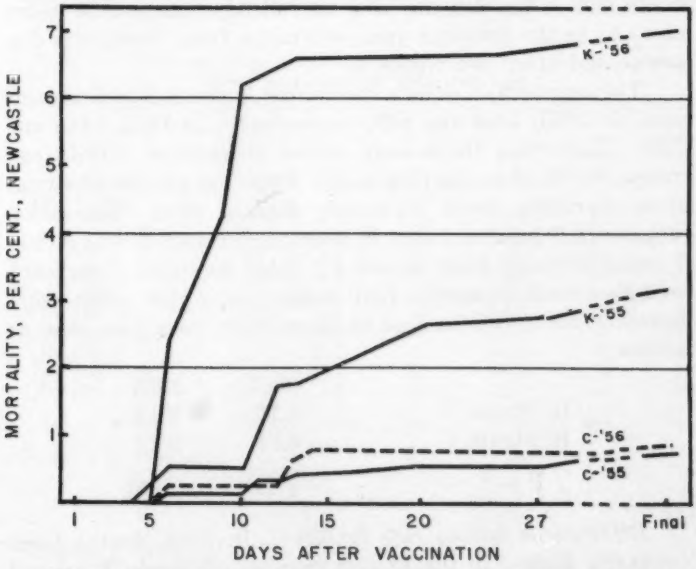

Fig. 1. Accumulative mortality from Newcastle disease following inoculation of 10- to 16-week-old pullets with wing-web vaccine. Deaths among pullets were significantly greater for Strain K than for Strain C in both 1955 and 1956.

Mortality due to Newcastle disease (Figure 1) ceased after two weeks, except for birds kept in colony houses and subsequently sacrificed because full recovery was not obtained. Morbidity was much greater than the mortality would indicate, but apparently many birds were able to recover fully. Some of those removed to colony houses were eventually returned to the flock, free of clinical symptoms of disease.

In 1956 the severe effects of the vaccination were evident four days thereafter. By that time some birds had died, numerous others showed nervous symptoms, and many more were obviously affected though not showing any paralysis. Autopsy on birds dead at 4 or 5 days, as well as those dying during the subsequent 24 hours, was not done until the 6th day after vaccination. This accounts for the apparent delayed reaction (Figure 1), compared to the experience of the previous year. However, the extent and severity of the reaction was obvious at the end of the 4th day and in full development 24 hours later. As in the previous year, mortality from Newcastle disease ceased after two weeks.

The numbers of pullets vaccinated in the K and C strains were: in 1955, 1200 and 847, respectively; in 1956, 1445 and 1389. Each year these were mixed at random, within age groups, on the same rearing range. From the graphs of cumulative mortality from Newcastle disease after vaccination (Figure 1) it is evident that in both years Strain K was stricken more severely than Strain C. Total mortality (percent) from Newcastle disease in four weeks, plus a few pullets subsequently destroyed because of permanent paralysis, was as follows:

	1955	*1956*
In Strain K	3.16	7.15
In Strain C	0.71	0.79
P =	<.001	<.001

Differences among sire families. In 1955, deaths from Newcastle disease in the 12 sire families of Strain K ranged from 0 to 5.2% and differences among them were not significant.

In the following year, with mortality over twice as high, deaths from Newcastle disease in 18 complete sire families ranged from 0 to 14.5% (Table 1). Because these were significantly higher among the 10-week-old pullets than among those 12 to 14 or 16 weeks of age, a direct comparison of all 18 sire families was not possible. Fourteen sires (cockerels previously untested) had daughters in only one of the three age groups, cited above. Four proven sires had daughters in each of these three periods and were represented proportionally in each.

Separate chi-square tests for homogeneity among the sire families in each age group showed a significant difference among them only in the pullets 12-14 weeks old. In the others the values for P were low (Table 1). However, the additive nature of chi-square values permits a pooling of all the data, without confounding due to age effects. When this is done, the chi-square value of 42.07 for 23 degrees of freedom indicates that there were significant differences (P = <.01) among

Table 1. Mortality from Newcastle disease, by sire family, among pullets vaccinated at different ages

	10 weeks		12-14 weeks		16 weeks	
	Pullets (no.)	Mort. (%)	Pullets (no.)	Mort. (%)	Pullets (no.)	Mort. (%)
Proven sires						
♂ K 1	31	0.0	84	3.6	35	0.0
♂ K 2	32	6.2	50	2.0	39	0.0
♂ K 3	38	13.2	84	2.4	26	3.8
♂ K 4	36	19.4	60	5.0	36	8.3
*Cockerels**	*Series A*		*Series B*		*Series C*	
In pen J 1	62	6.5	69	14.5	70	7.1
In pen J 2	58	13.8	—	—	61	0.0
In pen J 3	56	10.7	52	5.8	61	8.2
In pen J 4	70	11.4	72	8.3	75	9.3
In pen J 5	61	4.9	63	12.7	64	3.1
Totals & av.	444	9.68	534	6.74	467	4.92
Tests of homogeneity						
n	9		8		9	
df	8		7		8	
χ^2	11.95		16.48		13.64	
P	<.20		<.025		<.10	
Addition of chi-square						
df = 23	$\chi^2 = 42.07$			P = <.01		

* A given cockerel was used in only one series, being replaced by another for a subsequent series.

the sire families in mortality from Newcastle-disease virus following vaccination.

Among the proven sires (Table 1), which had daughters in all age groups, ♂ K 4 consistently showed greater losses than any of the other three. In contrast, ♂ K 1 showed a very low level of mortality.

In neither year were losses from Newcastle disease following vaccination high enough in the C strain to identify any differences among sire families.

Proof that genetic factors affect the resistance of pullets to Newcastle-disease vaccine was therefore provided by the difference, in each of two years, between the K and C strains in mortality attributable to the virus of Newcastle disease. Further evidence is seen in the highly significant differences among sire families of the K strain in 1956.

ADDITIONAL TESTS OF SUSCEPTIBILITY TO NCD VIRUS

It seemed desirable to test further for strain differences in resistance to Newcastle-disease virus and to do so at an earlier age. Cockerel chicks from pedigree matings of 1960 were used for such a study.

Through the courtesy of Dr. T. M. Goldhaft, Vineland Poultry Laboratories, Vineland, N. J., a vaccine was specially prepared from the same serial lot of virus as that used in 1956. The first series of cockerel chicks was reared to four weeks of age, to ensure loss of parental immunity, then divided at random into three lots per strain (K and C) of 42 to 44 chicks each. The virus preparation was suspended in physiological saline and injected into the pectoral muscle. For the standard dose, as recommended by the manufacturer, each chick actually received the equivalent of .01 cc of original (chorio-allantoic) fluid from embryonating chicken eggs infected with Newcastle-disease virus, brought to a volume of 0.2 cc by the use of saline. The other dose levels were 10 an 25 times the standard, each brought to a volume of 0.2 cc by the use of saline. Only one chick (K strain, on lowest dose) died during the following two weeks, at which time the test was terminated.

A second lot of cockerel chicks, 12 days of age, was vaccinated using only 5 times the standard level of virus. In this test, mortality, which started on the 5th day after vaccination and continued for only 5 days, was as follows:

	Strain K	*Strain C*
Chicks inoculated:	247	215
Died, number:	18	1
Died, percent:	7.3	0.5

The differences between strains is highly significant. These chicks had been pedigreed as to sires, and among the sire families losses ranged from 0 to 20%. A chi-square test for homogeneity among the 6 sire families in Strain K yielded a value of chi square of 20.7 and for P of $<.001$, which indicates significant differences among these 6 sires in susceptibility of their progeny to Newcastle-disease virus.

Altogether the results of this test with young cockerels show differences between strains, and among sire families in Strain K, that are very similar to those observed in pullets at 10 to 16 weeks of age after vaccination in 1956.

The question arises why chicks of the second series should have proven more susceptible to a dose of 5 × standard at 12 days of age than did those of the first series to doses of 1 ×; 10×; and 25× standard at 28 days of age. Such results were to be expected on the simple ground of greater susceptibility at earlier ages if there were no passive immunity. On the other hand, if there had been passive immunity from egg-borne antibodies giving protection to newly hatched chicks, but wearing thin by (normally) four weeks of age, one would expect the older chicks to be more susceptible.

In this case 40% of the hens that produced the cockerel chicks had been vaccinated as pullets with wing-web vaccine against Newcastle disease and the others had not. All of them had been reared together with birds that were vaccinated. The former group might have transmitted protective antibodies; presumably the latter could not. The best explanation may be

the simple one that chicks are more susceptible to Newcastle-disease virus at 12 days than at 28 days of age.

The fact that young birds are more susceptible than older ones was clearly shown by the pullets of Strain K inoculated at different ages in 1956. Among these, mortality from Newcastle disease (in daughters of the same four proven sires) was 10.2% in those at 10 weeks but only 3.2% at 12–14 weeks and 2.9% at 16 weeks.

DISCUSSION

The clear-cut differences between our K and C strains in mortality after vaccination against Newcastle disease in 1955 and 1956 are considered evidence that these strains differ genetically in ability to resist the Roakin strain of the Newcastle-disease virus. As the Roakin strain is a comparatively virulent form, it seems probable that the two strains of Leghorns should differ also in abiiity to withstand other and less virulent strains of the Newcastle virus.

This evidence, coupled with the significant differences among sire families of Strain K in mortality following experimental inoculation, suggests (to the authors, at least) that, by selective breeding, resistance to the mild form (or forms) of Newcastle disease might be raised to a level at which losses would be negligible. Other controls that have been tried include (a) attempts to eradicate, and (b) vaccination. Attempts to eradicate Newcastle disease ("fowl pest") by slaughtering infected flocks have been unsuccessful in Great Britain, where, after 11 long years of that official policy, more hens were slaughtered in the twelfth year (1959) than ever before. Control by vaccination is effective, but some poultrymen (including the authors of this paper) have stopped vaccinating because Newcastle disease does not seem serious enough in their flocks to justify the time and expense involved.

The fact that reactors to tests for antibodies against Newcastle-disease virus are commonly found in healthy birds —these in healthy flocks laying well and not known to have experienced the disease—suggests that most birds are so highly resistant to mild strains of the Newcastle virus that when infected they never show the nervous disorders sometimes

caused by that virus. For most laying birds the usual visible sign of infection is a temporary cessation of laying. Some birds do not stop at all, and others resume laying in a few days, but some may not do so for several weeks.

With such variation available, and our evidence of genetic differences in resistance, the authors believe that natural selection would eventually raise the level of resistance in breeding flocks regularly exposed, but that the breeder of pedigreed flocks could accelerate that process by appropriate progeny testing. Significant differences in mortality among sire families, as found in our Strain K in 1956 and in the cockerel chicks tested in 1960, provide ample material for selection. The breeder's greatest problem in breeding resistant stock may be to ensure adequate exposure, through natural channels of infection, at a suitable age. The desirability and feasibility of utilizing genetic resistance as a method of controlling specific diseases and of enhancing it through proper breeding procedures have been discussed elsewhere.[3]

SUMMARY

In two successive years, inoculation of over 2,000 White Leghorn pullets at 10 to 16 weeks of age with Roakin strain, wing-web, live-virus vaccine against Newcastle disease caused significant immediate mortality from that disease. One strain of fowls was susceptible while another was comparatively resistant. Mortality from Newcastle disease immediately following inoculation was 3.2 and 7.2% in Strain K, against 0.7% and 0.8% for Strain C, in 1955 and 1956, respectively.

In a subsequent test, with over 200 cockerel chicks of each strain, mortality from Newcastle disease was 7.3% in Strain K, but only 0.5% in Strain C.

Significant differences in susceptibility among sire families within Strain K were observed in pullets in 1956 and in the cockerel chicks.

The possibilities of control by breeding resistant stock are briefly discussed.

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FURALTADONE WATER MEDICATION: ITS USE IN AVIAN SALMONELLOSIS

BERT W. BIERER, V.M.D., H. D. VALENTINE, V.M.D., and C. L. VICKERS, D.V.M.*

Received 28 Nov 1960

THE use of nitrofurans in Salmonellosis has been briefly reviewed by a number of workers.[1,3,4,5,6,7] Furazolidone, used extensively for a number of years, is of limited usefulness because it is insoluble in water. Water medication can be quickly and easily applied, and feed does not have to be removed from hoppers.

In experimentally induced fowl typhoid and infections with *S. typhimurium* and *S. heidelberg*, furaltadone in water was medically equivalent to furazolidone in feed.[2] Therefore, experiments were instituted to determine the effect of various levels of furaltadone administered in water on artificially induced pullorum disease, fowl typhoid, and *S. typhimurium* infection.

MATERIALS AND METHODS

Expt. 1. Battery cages, each containing 25 chicks, were arranged in experimental design as indicated in Table 1. Brooder temperatures were maintained at 90°F. At 20 hours of age all groups scheduled to be infected were given oral

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doses of about 150 million *S. pullorum* (½ cc of a 24-hour-old broth culture adjusted to an optical density of 0.25 on Bausch and Lomb Spectronic 20 colorimeter, the equivalent of No. 1 on McFarland scale). Prophylactic medication was present when chicks were placed in the pens, and therapeutic medication was applied when clinical evidence of infection (mortality, chill, and soiled vents) was observed. Medication was continued until 10 days of age, when the experiment was terminated. Feed consumed and average body weights per pen were recorded . The cause of all deaths during the experiment was established by routine diagnostic procedures. Chicks and poults used in this and the following-described experiments were from hatcheries with no recent history of Salmonella

Table 1. Furaltadone water medication mortality results.

Experiment:	1. *S. pullorum* Chicks	2. *S. gallinarum* Chicks	3. *S. gallinarum* Poults	4. *S. typhimurium* Poults
Total birds per experiment	250	250	100	100
Birds per pen	25	25	10	10
Prophylactic medication	% died	% died	% died	% died
1.00 g/gal.	4	0	0	20
0.50 g/gal.	0	0	0	20
0.25 g/gal.	8	0	20	30
Number birds died prior to therapeutic medication	3	12	2	5
Therapeutic medication	% survivors died post-medication			
1.00 g/gal.	26	26	0	80
0.50 g/gal.	16	30	11	57
0.25 g/gal.	48	63	20	10
Infected unmedicated	% died	% died	% died	% died
(Replicates)	72	100	90	70
	80	100	60	80
Uninfected unmedicated	% died	% died	% died	% died
(Replicates)	4	0	10	0
	0	0	0	10

All experiments terminated 10 days after infection.

infection. Complete feed, not requiring supplementation and free of coccidiostats, antibiotics, and chemical additives, was used. No disinfectants were used, and water medication, given in metal-base glass gallon waterers, was changed daily.

Expt. 2. This was a replicate to Expt. 1 except that exposure was about 150 million *S. gallinarum* (see Table 1).

Expt. 3. This was a replicate to Expt. 1 except that 10 poults per cage were used, brooder temperatures were maintained at 95°F, and an oral dose of about 75 million *S. gallinarum* was given at 36 hours of age.

Expt. 4. Ten poults per pen were placed in the experimental design indicated in Table 1. At 24 hours of age, scheduled infected groups were given oral doses of about 75 million *S. typhimurium*. Brooders were maintained at 95°F. Other details were as in Expts. 1, 2, and 3.

RESULTS AND DISCUSSION

Furaltadone prophylactic water medication at 0.25 g/gal. of drinking water resulted in a marked reduction in mortality in chicks with *S. pullorum* infection, a complete control of mortality in chicks with *S. gallinarum* infection, and a marked reduction in mortality in poults with *S. typhimurium* and *S. gallinarum* infections. Higher levels of prophylactic medication resulted in some greater control of mortality (see Table 1).

Furaltadone therapeutic medication resulted in a reduction in mortality in all four experiments at 0.25 g/gal. but in three of the four experiments resulted in a *marked* reduction in mortality when given at 0.5 g/gal. In *S. typhimurium* infection in poults the mortality was greater at 0.5 and 1 g/gal. therapeutic levels. The cause of this unusual result could not be determined, but erratic results with medicated infected poults are not unusual.

In general, average body weights for all medicated groups were about the same and compared favorably with uninfected unmedicated controls. In most instances, surviving unmedicated controls were lower in body weights than medicated groups.

While all survivors in all four trials were necropsied, mortality and average body weights were adequate for an evaluation of furaltadone water medication dosage. The value of this drug in reducing the carrier state has already been reported.[2] A study of the medicated necropsied birds as compared to unmedicated infected controls, in all four trials, revealed that furaltadone water medication prevented the progress of the disease prophylactically and arrested the progress therapeutically. In general these effects were most pronounced on prophylactic levels of 0.25 g/gal. and above, and on therapeutic levels of 0.5 g/gal. and above—the same drug levels most effective in reducing mortality.

The infections induced in these studies were more severe than one would expect under natural field conditions, since all birds were infected simultaneously, and those in the therapeutic groups were not treated until the disease was in advanced stages.

SUMMARY AND CONCLUSIONS

1. Prophylactic water furaltadone medication at 0.25 g/gal. resulted in a marked reduction in mortality in pullorum disease in chicks, fowl typhoid in chicks and poults, and *S. typhimurium* infection in poults.

2. Therapeutic medication at 0.25 g/gal. resulted in a reduction in mortality in all four trials. However, mortality reduction was marked on the 0.5 g/gal. level in 3 of 4 trials but erratic results were obtained in poults with *S. typhimurium* therapeutic medication.

3. In general, average body weights of all medicated groups at 10 days compared favorably with uninfected unmedicated controls, but were lower in the infected unmedicated groups.

4. Further evaluation of furaltadone water medication in more extensive floor pen trials, under field conditions, is indicated.

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CASE REPORT: A SYNOVITIS CAUSED BY A STRAIN OF *ESCHERICHIA COLI*

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Received 14 Mar 1961

On December 2, 1959, a consignment of 4-week-old White Rock pullets was received from North Carolina. Of a group of 2500 birds 300 had been reported to be lost. Necropsy of 2 birds revealed synovitis of most joints, a large caseous mass in the region of the left kidney of one bird, and three spherical caseous masses, 3-4 mm. in diameter, in the liver of the other bird. A strain of *Escherichia coli* was isolated from the kidney.

This isolate was found to belong to "O" group 15 by the Communicable Disease Center of the United States Public Health Service, Chamblee, Georgia.

MATERIALS AND METHODS

The chickens used in these experiments were White Leghorns from PPLO-free unvaccinated parents. They were housed in brooders or batteries in isolation rooms before and during these experiments.

One-tenth ml of a 24-hr. tryptose broth culture of this strain of *E. coli* was inoculated into 5-week-old chickens by intravenous, air-sac, or intramuscular routes. In a 2-week observation period no lesions developed in 20/20 birds inoculated into the air sac and 12/12 inoculated intramusclarly. All 12 inoculated intravenously developed joint infections. Five of these birds also developed caseous lesions around the kidney. The lesions appeared to be an extension of a hip joint infection that had spread under the capsule of the kidney. Two birds developed extensive pockets of infection between the deep and superficial pectoral muscles. These infections appeared to be extensions of the shoulder-joint lesions. Subsequent experiments indicated that the lesions healed spontaneously in 3–4 weeks.

In a separate experiment, pour-plate cultures were made from 0.2 ml of blood of birds 1, 2, 5, and 8 days after intravenous inoculations. These respective cultures were positive in 9/10, 7/10, 6/10, and 0/10 of the birds. The number of bacteria per 0.2-ml sample ranged between 2 and 500 one day after inoculation, and between 5 and 24 five days after inoculation.

The lesions observed in the 3 similar experiments are recorded in Table 1. These results indicate the joint involved most frequently is the knee (tibular-femoral) joint. Kidney

Table 1. Distribution of lesions following intravenous inoculation of chickens with *Escherichia coli* belonging to "O" group 15

	Joints infected						Lesions of:		Neg/ birds inoculated
	Knee	Elbow	Shoulder	Hock	Wrists	Hip	Kidney	Pectoral muscle	
Exp. 1	20	10	8	8	2	5	5	2	0/12
Exp. 2	19	9	8	3	2	0	0	0	0/12
Exp. 3	18	8	4	7	1	0	0	0	1/10
Total	57	27	20	18	5	5	5	2	1/34

and pectoral muscle infections were not reproduced after the first experiment.

DISCUSSION

Synovitis produced by *E. coli* may be confused with staphylococcal synovitis[1] and infectious synovitis.[2] Differentiation can easily be made by culturing the infected material and identifying the agent. To differentiate the pathogenic coliform ("O" group 15) from contaminants, serotyping should be done. It is interesting to note that staphylococcal synovitis and infectious synovitis chiefly involved the hock and joints. In coliform synovitis the knee, elbow (humerus-radius-ulnar), and shoulder joints are all involved more frequently than the hock and foot joints. Blood culture findings indicate that joint lesions appear subsequent to an earlier bacteriemia. Since the birds tend to recover spontaneously in about 3–4 weeks, many treatments might appear to be successful. Failure of the air-sac route of inoculation to produce pericarditis differentiates this coliform from those associated with "air sac disease."

CONCLUSION

An isolate of *Escherichia coli* belonging to "O" group 15 was isolated from a naturally occurring synovitis of chickens. The condition was reproduced by a tryptose broth culture inoculated intravenously, but not intramuscularly or via air sac.

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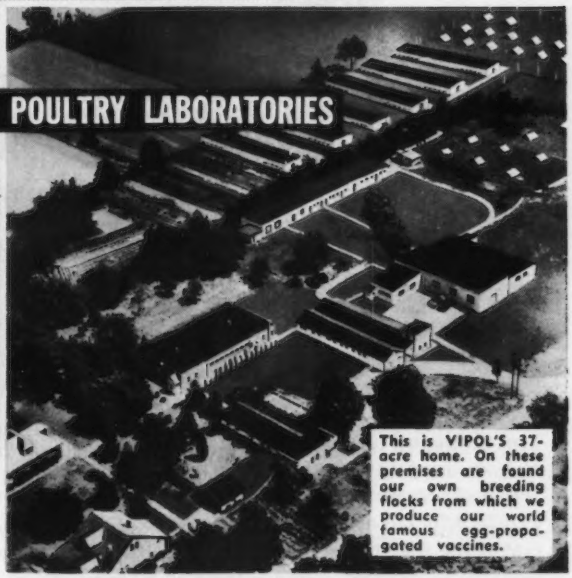

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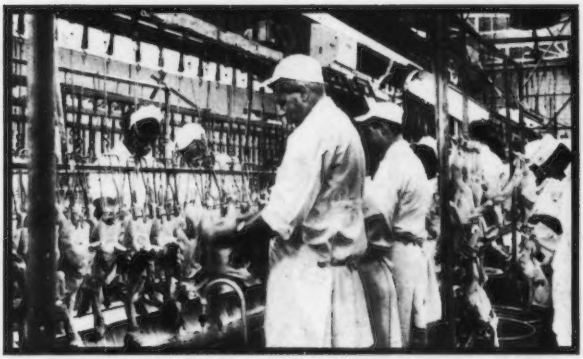

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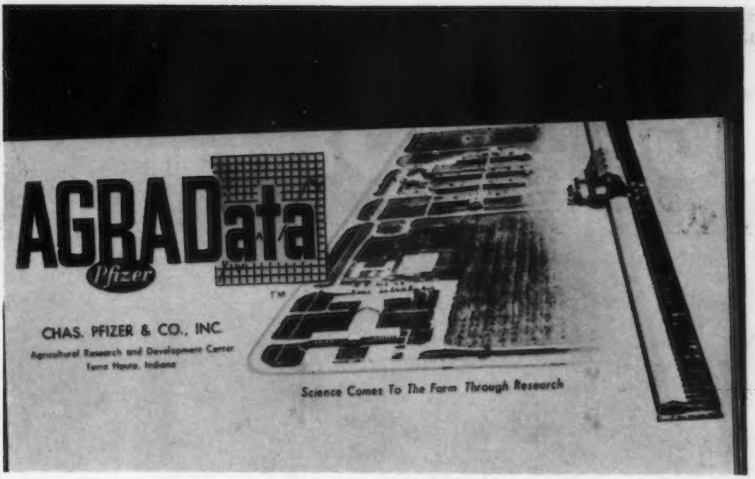

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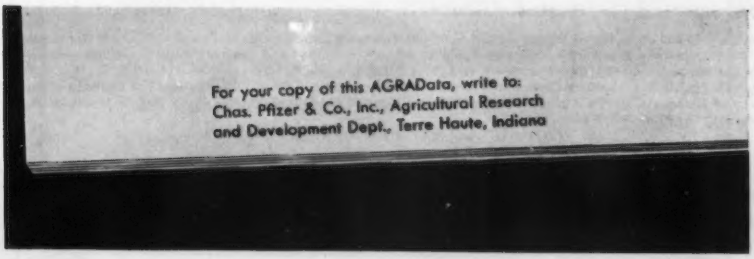
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Once again Dr. Salsbury's Histostat-50 has been proven to prevent blackhead best and to provide other benefits needed by turkeys for fast, profitable gains. The following is taken from the "1959 TURKEY DISEASE CONTROL REPORT," University of Minnesota, Northwest School and Experiment Station, located at Crookston, Minnesota. It is reprinted here to give you factual data on a series of tests that are important to everyone connected with the turkey industry.

WEIGHT, FEED EFFICIENCY, FINISH, AND MORTALITY DATA

Lot Number	1B	2B	3B	4B	5B	6B	7B	8B
Drug Treatment	None	1 lb. Histostat 50	1lb.11oz. Hepzide 30	2 lb. 5 oz Hepzide 30	2 lb. NF 64	3 lb. NF 64	3 lb. NF 179	2 lb. NF 180
Average Weight Toms, Lbs.	21.96	22.94	21.81	21.95	22.47	21.49	23.44	23.05
Average Weight Hens, Lbs.	15.13	15.58	14.99	15.01	15.72	15.28	15.54	14.74
Feed Required Per Lb. Gain	5.08	4.17	4.67	4.32	4.63	3.91	3.98	4.48
Feathering—Toms.	93.75	98.75	94.44	100.00	100.00	89.29	98.96	97.50
Hens	100.00	100.00	100.00	100.00	98.75	98.86	100.00	99.00
Fleshing —Toms.	84.38	96.25	90.28	84.38	92.30	80.95	92.71	88.75
Hens	100.00	100.00	100.00	100.00	98.75	98.86	100.00	98.00
Mortality Blackhead,%	24%	2%	12%	10%	22%	14%	4%	12%

Please note the consistent superiority of lot 2B (Histostat-50). This lot had the *lowest death loss*, and was *highest of all the commercially available drugs tested on weight gains, feed efficiency*, and *fleshing*.

As an educational staff member or research worker, you may wish further information about Histostat-50 or some other of Dr. Salsbury's many fine products or services. Please phone, write, or wire. Your inquiry will get a prompt reply.

Dr. SALSBURY'S LABORATORIES
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